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(54) Title: NOVEL N-ACETYL GALACTOSAMINE TRANSFERASES AND NUCLEIC ACIDS ENCODING THE SAME

(57) Abstract: An enzyme which transfers N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage was isolated and the structure of its gene was explained. This led to the production of said enzyme or the like by genetic engineering techniques, the production of oligosaccharides using said enzyme, and the diagnosis of diseases on the basis of said gene or the like. The present invention uses a protein having the amino acid sequence shown in SEQ ID NO: 1, 3, 26 or 27 in the Sequence Listing or a variant of said amino acid sequence wherein one or more acids are substituted or deleted, or one or more acids are inserted or added and having the activity of transferring N-acetylgalactosamine (GalNAc) to N-acetylglucosamine serving as a substrate via a  $\beta$ 1-4 linkage and nucleic acids encoding said protein.

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## DESCRIPTION

Novel N-Acetylgalactosamine Transferases and  
Nucleic Acids Encoding the Same5 Technical Field

The present invention relates to novel enzymes having the activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage and nucleic acids encoding the same, as well as to nucleic acids for assaying  
10 said nucleic acids.

Background Art

In various kinds of organisms, structures having a linkage of disaccharide of N-acetylgalactosamine-N-  
15 acetylglucosamine have been found in oligosaccharides of glycoproteins and glycolipids [see References 1 and 2]. In humans, this disaccharide structure is known as a  $\beta$ 1-4 linkage (GalNAc $\beta$ 1-4GlcNAc), and is found only in N-glycans [see Reference 3]. Methods for obtaining human-type  
20 oligosaccharides including said structure are limited to methods using complicated chemical synthesis and methods obtaining the oligosaccharides from natural proteins. Further, the above disaccharide structure includes in vivo a galactose substituted for a N-acetylgalactosamine.  
25 Therefore, it is a lengthy, laborious process to obtain oligosaccharides having the target disaccharide structure.

Prior to the present application, the inventors identified ppGalNAc-T10, -T11, -T12, -T13, -T14, -T15, -T16, -T17, CSGalNAc-T1, and -T2 as enzymes having an activity of

transferring N-acetylgalactosamine to glucuronic acids and polypeptides, and further, they clarified the structures of these genes. Already known are at least 22 N-acetylgalactosamine transferases that have the activity of  
5 transferring N-acetylgalactosamine (Table 1), and each of the transferases have different specificities of acceptor substrates.

Table 1 N-acetylgalactosamine transferase and the substrate specificity

Formal Name	Abbreviation	Origin	Substrate specificity	References
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase I	ppGalNAc-T1	human	Ser/Thr	White, T. etc (1995)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase II	ppGalNAc-T2	human	Ser/Thr	White, T. etc (1995)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase III	ppGalNAc-T3	human	Ser/Thr	Bennet, B. P. etc (1996)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase IV	ppGalNAc-T4	human	Ser/Thr	Bennet, B. P. etc (1998)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase VI	ppGalNAc-T6	human	Ser/Thr	Bennet, B. P. etc (1999) (1)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase VII	ppGalNAc-T7	human	Ser/Thr	Bennet, B. P. etc (1999) (2)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase VIII	ppGalNAc-T8	human	Ser/Thr	Walte, K. E. etc (2000)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase IX	ppGalNAc-T9	human	Ser/Thr	Toba, S. etc (2000)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase X	ppGalNAc-T10	human	Ser/Thr	JP No. 2001-401455 (unpublished)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XI	ppGalNAc-T11	human	Ser/Thr	JP No. 2001-401507 (unpublished)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XII	ppGalNAc-T12	human	Ser/Thr	JP No. 2001-401507 (unpublished)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XIII	ppGalNAc-T13	human	Ser/Thr	JP No. 2001-401507 (unpublished)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XIV	ppGalNAc-T14	human	Ser/Thr	Guo, J. M. etc (2002)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XV	ppGalNAc-T15	human	Ser/Thr	JP No. 2001-401507 (unpublished)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XVI	ppGalNAc-T16	human	Ser/Thr	JP No. 2001-401507 (unpublished)
UDP-GalNAc:polypeptide N-acetylgalactosaminyl transferase XVII	ppGalNAc-T17	human	Ser/Thr	JP No. 2001-401507 (unpublished)
$\beta$ 1,4-N-acetylgalactosamine transferase	$\beta$ 4GalNAcT	human	GM3, GM3, LacCer	Nagata, Y. etc (1992)
UDP-GalNAc:H- $\alpha$ 1,3-N-acetylgalactosamine transferase	Hist blood A group transferase	human	Fuc $\alpha$ 1,2Gal $\beta$ 1-R	Yamamoto, P. etc (1990)
UDP-GalNAc:globoside $\alpha$ 1,3-N-acetylgalactosamine transferase I	formalin glycolipid synthase	human	GalNAc $\beta$ 1-3Gal $\alpha$ 1 -4Gal $\beta$ 1-3Glc-Cer	Xu, H. etc (1999)
Chondroitin sulfate N-acetylgalactosaminyl transferase I	CSGalNAc-T1	human	GlcA	JP No. 2002-129156 (unpublished)
Chondroitin sulfate N-acetylgalactosaminyl transferase II	CSGalNAc-T2	human	GlcA	JP No. 2002-24202 (unpublished)

Disclosure of Invention

Isolation of an enzyme having the activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage and an explanation of the structure of its gene enable the production of said enzyme or the like through genetic engineering techniques, and the diagnosis of diseases on the basis of said gene or the like. However, such an enzyme has not been isolated/purified yet and there is no key to isolating such an enzyme and identifying its gene. Therefore, no antibody against such an enzyme has been prepared.

Therefore, the present invention provides a protein having an activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage and nucleic acids for encoding the same. The present invention also provides a cell introduced with a recombinant vector expressing said nucleic acids in a host cell and said nucleic acids, and expressing said nucleic acids and said proteins. Further, said protein expressed can be used for producing an antibody. Therefore, the present invention also provides a method for producing said protein. Further, the expressed protein and said antibody to the protein can be applied to immunochemical staining, and immunoassay of RIA and EIA and the like. Moreover, the present invention provides an analytical nucleic acid for assaying the above nucleic acid of the present invention.

As described above, the objective enzymes have not yet been identified, and therefore, the partial sequence of the amino acids cannot be informed. In general, it is

difficult to isolate and purify proteins which are included in only a very small quantity in cells. Therefore, it is supposed that it is not easy to isolate enzymes which have so far not been isolated from cells. Thereat, the inventors

5 tried to isolate and purify target enzymes, by making a region of which identity is thought to be high into a target, which may have the homologous sequence in nucleic acid sequences of genes between a objective enzyme and various kinds of enzymes having relatively similar activity.

10 Specifically, the inventors first searched nucleic acid sequences of publicly-known  $\beta$ 1,4-galactose transferases, and identified homologous regions. Second, primers were designed based on these homologous regions, and a full-length open reading frame was identified from cDNA library by

15 5' RACE (rapid amplification of cDNA ends) method. Further, the inventors succeeded in cloning a gene of said enzyme by PCR, and completed the present invention by determining nucleic acid sequences thereof and putative amino acid sequences.

20 The present invention provides a protein having the activity of transferring N-acetylgalactosamine and nucleic acid encoding the same, and thereby assists in satisfying these various requirements in the art.

Namely, the present invention provides a mammal

25 protein having the activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage.

The human protein of the present invention has, typically, amino acid sequence of SEQ ID NO: 1 or 3, which

is presumed from nucleic acid sequence of SEQ ID NO: 2 or 4.

The mouse protein of the present invention has amino acid sequence of SEQ ID NO: 26 or 28, which is presumed from nucleic acid sequence of SEQ ID NO: 27 or 29.

5           The present invention includes not only the protein having the amino acid sequence which is selected from a group consisting of SEQ ID NOs: 1, 3, 26 and 28 but also proteins having an identity of 50 % or more to said sequence. The present invention includes proteins having said amino  
10 acid sequence, wherein one or more amino acids are substituted or deleted, or one or more amino acids are inserted or added.

          The proteins of the present invention have amino acid sequences which have an identity of 60 % or more, preferably  
15 70 % or more, more preferably 80 % or more, still more preferably 90 %, and most preferably 95 % to the amino acid sequence which is selected from a group consisting of SEQ ID NOs: 1, 3, 26 and 28.

          The present invention provides nucleic acids encoding  
20 the protein of the present invention.

          The nucleic acids of the present invention have, typically, the nucleic acid sequence which is selected from a group consisting of SEQ ID NOs: 2, 4, 27 and 29, nucleic acid sequences in which one or more nucleic acids are  
25 substituted, deleted, inserted and/or added to the above nucleic acid sequence, or a nucleic acid sequence which hybridizes with said nucleic acid sequence under stringent conditions, and which includes the nucleic acids complementary to the above sequences. In one embodiment,

the present invention includes, but is not limited to, nucleic acids having the nucleic acid sequence represented by nucleotides 1-3120 of the nucleic acid sequence shown in SEQ ID NO: 2, nucleotides 1-2997 of the nucleic acid

5 sequence shown in SEQ ID No: 4, nucleotides 1-3105 of the nucleic acid sequence shown in SEQ ID NO: 27, nucleotides 1-2961 of the nucleic acid sequence shown in SEQ ID No: 29.

The present invention provides a recombinant vector containing the nucleic acids of the present invention.

10 The present invention provides the transformants obtained by introducing the recombinant vector of the present invention into host cells.

The present invention provides an analytical nucleic acid which hybridizes to the nucleic acids encoding the protein of the present invention under stringent conditions. 15 The analytical nucleic acid preferably has the sequence shown in any one of SEQ ID NOs: 20, 21, 23 and 24 in the case of using the analytical nucleic acid of the present invention as a probe for assaying the nucleic acids encoding said protein. Further, the analytical nucleic acid of the 20 present invention can be used as a cancer marker.

The present invention provides an assay kit comprising the analytical nucleic acid which hybridizes to the nucleic acid of the present invention.

25 The present invention provides the isolated antibody binding to the protein of the present invention or the monoclonal antibody thereof.

Further, the present invention provides a method for determining a canceration of biological sample which



comprises a step of quantifying the protein or the nucleic acid of the present invention in the biological sample.

#### Brief Description of Drawings

5           Fig. 1 is a graph showing the quantitative analysis of expression level of NGalNAc-T1 or NGalNAc-T2 gene in various human tissues by the real time PCR. The axis of ordinates represents a relative ratio of expression level of NGalNAc-T1 or NGalNAc-T2 gene to that of a control  
10   glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The expressions of NGalNAc-T1 and NGalNAc-T2 gene are represented as a black bar and a white bar, respectively.

          Fig. 2 is a graph showing the quantitative analysis of expression level of NGalNAc-T1 (panel A) or NGalNAc-T2  
15   (panel B) gene in human lung cancerous tissue and normal tissue by the real time PCR. The axis of ordinates represents a relative ratio of expression level of NGalNAc-T1 or NGalNAc-T2 gene to that of a control human  $\beta$ -actin gene. The axis of abscissas represents numbers relating to  
20   each patient. The normal tissue and the cancerous tissue are represented as a white bar and a black bar, respectively.

          Fig. 3 shows LacdiNAc synthesizing activity of NGalNAc-T2 toward asialo/agalacto-fetal calf fetuin. The asialo/agalacto-FCF appears as approximately 55 and 60 kDa  
25   band (lane 1). The NGalNAc-T2 effectively transfers GalNAc to asialo/agalacto-FCF (lane 5). The band mostly disappeared by GPF treatment (lane 6).

          Fig. 4 shows an analysis of N-glycan structures of glycodelin from NGalNAc-T1 and NGalNAc-T2 gene transfected

CHO cells. The non-reducing terminal GalNAc is detected only when NGalNAc-T1 or NGalNAc-T2 gene is co-transfected with glycodeclin gene.

Fig. 5 shows one-dimensional  $^1\text{H}$  NMR spectrum of the structure of GalNAcb1-4GlcNAc-O-Bz produced by NGalNAc-T2.

Fig. 6 shows two-dimensional  $^1\text{H}$  NMR spectrum of the structure of GalNAcb1-4GlcNAc-O-Bz produced by NGalNAc-T2.

#### Detailed Description of the Invention

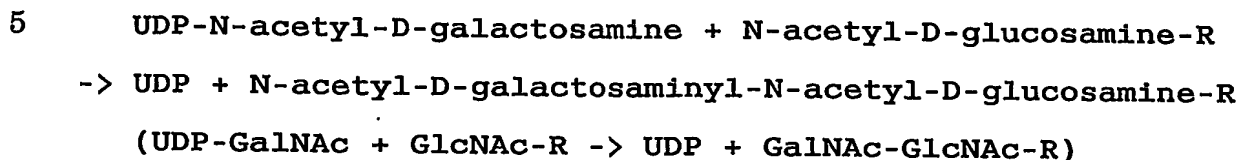
In order to explain the present invention, a preferable embodiments for carrying out the invention are described in detail below.

##### (1) Proteins

The nucleic acid encoding the human protein of the present invention cloned by the method described in detail in the examples below has the nucleotide sequence shown in SEQ ID NO: 2 or 4 in the Sequence Listing under which a deduced amino acid sequence encoded thereby is also shown. In addition, SEQ ID NO: 1 or 3 shows only said amino acid sequence.

The proteins (hereinafter, denominated "NGalNAc-T1" and "NGalNAc-T2") of the present invention obtained in the examples below are enzymes having the properties listed below. In addition, each property of the proteins of the present invention and the method for determining the activity thereof are described in detail in the examples below.

Activity: Transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage. The catalytic reaction is represented by the reaction formula:



Specific substrate: N-acetyl-glucosamine such as N-  
10    acetylglucosamine  $\beta$ 1-3-R (R is a residue of which hydroxyl group of mannose and p-nitrophenol and the like binds via an ether linkage).

      In a preferable embodiment, the proteins of the  
15    present invention have at least one of the following properties, preferably these properties:

(A) Specificity of acceptor substrates

      (a) When O-linked oligosaccharides are used as an acceptor substrate, said proteins have the activity of  
20    transferring N-acetylgalactosamine to GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$ -pNp (hereinafter, "core2-pNp"), GlcNAc $\beta$ 1-3GalNAc $\alpha$ -pNp (hereinafter, "core3-pNp"), GlcNAc $\beta$ 1-6GalNAc $\alpha$ -pNp (hereinafter, "core6-pNp") via a  $\beta$ 1-4 linkage, wherein the abbreviations used are: GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; Gal, galactose; pNp, p-nitrophenyl.  
25    Preferably, said proteins have the transferring activity to core6-pNp.

      (b) When N-linked oligosaccharides are used as an acceptor substrate, said proteins have the activity of

transferring N-acetylgalactosamine to GlcNAc at the non-reducing end of said oligosaccharides via a  $\beta$ 1-4 linkage, provided that said activity reduces when said oligosaccharides have the following properties:

5 (i) having fucose (Fuc) residues in the structure of said oligosaccharides; and

(ii) having one or more branched chains wherein GalNAc residues bind to GlcNAc residues at the non-reducing end.

10 (B) Optimum pH in enzymatic activity

The activity tends to be higher in pH 6.5 of MES (2-morpholineethanesulfonic acid) buffer. In HEPES ([4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer, the activity tends to be higher in pH 6.75 for NGalNAc-T1 and pH 15 7.4 for NGalNAc-T2.

(C) Requirement of divalent ions

In NGalNAc-T1, the activity tends to be higher in the MES buffer including at least  $Mn^{2+}$ , or  $Cu^{2+}$ , preferably  $Mn^{2+}$ . In NGalNAc-T2, the activity tends to be higher in the MES 20 buffer including  $Mg^{2+}$ ,  $Mn^{2+}$ , or  $Co^{2+}$ , preferably  $Mg^{2+}$ .

The nucleic acid encoding the mouse protein of the present invention also has the nucleotide sequence shown in SEQ ID NO: 27 or 29 in the Sequence Listing under which a 25 deduced amino acid sequence encoded thereby is also shown. In addition, SEQ ID NO: 1 or 3 shows only said amino acid sequence. The proteins (hereinafter, denominated "mNGalNAc-T1" and "mNGalNAc-T2") of the present invention are enzymes having the above properties.

The present invention provides a protein having an activity for transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage. So far as the proteins of the present invention have the properties described herein, the origins thereof and the method for producing them and the like are not limited. Namely, the proteins of the present invention include, for example, native proteins, proteins expressed from recombinant DNA using genetic engineering techniques, and chemically synthesized proteins.

The protein of the present invention has typically an amino acid sequence consisting of 1039 amino acids shown in SEQ ID NO: 1, 998 amino acids shown in SEQ ID NO: 3, 1034 amino acids shown in SEQ ID NO: 26, or 986 amino acids shown in SEQ ID NO: 28. However, it is well-known that in native proteins, there are mutant proteins having one or more variants of amino acids, depending on a mutation of gene based on various species of organisms which produce the proteins, and various ecotypes, or a presence of very similar isozymes or the like. In addition, the term "mutant protein(s)" used herein means proteins and the like having a variant of said amino acid sequence, wherein one or more amino acids are substituted or deleted, or one or more amino acids are inserted or added in the amino acid sequence of SEQ ID NO: 1, 3, 26 or 28, and having the activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage. The expression "one or more" here preferably means 1-300, more preferably 1-100, and most

preferably 1-50. Generally, in the instance that amino acids are substituted by site-specific variation, the number of amino acids that can be substituted to the extent that the activity of the original protein can be retained is preferably 1-10.

Proteins of the present invention have the amino acid sequences of SEQ ID NO: 1 or 3 and SEQ ID NO: 2 or 4 (lower), or amino acid sequences of SEQ ID NO: 26 or 28 and SEQ ID NO: 27 or 29 (lower) based on the premise of nucleotide sequences of the cloned nucleic acids, but are not exclusively limited to the proteins having these sequences, and are intended to include all homologous proteins having the characteristics described herein. The identity is at least 50 % or more, preferably 60 %, more preferably 70 % or more, even more preferably 80 % or more, still more preferably 90 % or more, and most preferably 95 % or more.

As used herein, the percentage identity of amino acid sequences can be determined by comparison with sequence information using, for example, the BLAST program described by Altschul et al. (Nucl. Acids. Res. 25, pp. 3389-3402, 1997) or the FASTA program described by Pearson et al. (Proc. Natl. Acad. Sci. USA, pp. 2444-2448, 1988). These programs are available from the website of National Center for Biotechnology Information (NCBI) or DNA Data Bank of Japan (DDBJ) on the Internet. Various conditions (parameters) for homology searches with each program are described in detail on the site, and searches are normally performed with default values though some settings may be appropriately changed. Other programs used by those skilled in the art of

sequence comparison may also be used.

Generally, a modified protein containing a change from one amino acid to another amino acid having similar properties (such as a change from a hydrophobic amino acid to another hydrophobic amino acid, a change from a hydrophilic amino acid to another hydrophilic amino acid, a change from an acidic amino acid to another acidic amino acid or a change from a basic amino acid to another basic amino acid) often has similar properties to those of the original protein. Methods for preparing such a recombinant protein having a desired variation using genetic engineering techniques are well known to those skilled in the art and such modified proteins are also included in the scope of the present invention.

Proteins of the present invention can be obtained in bulk by, for example, introducing and expressing the DNA sequence of SEQ ID NO: 2, 4, 27 or 29 representing a nucleic acid of the present invention in *E. coli*, yeast, insect or animal cells using an expression vector capable of being amplified in each host, as described in the examples below.

When the identity search of the protein of the present invention is performed using GENETYX (Genetyx Co.), the NGalNAc-T1 has 47.2 % identity to NGalNAc-T2, 84.3 % identity to mNGalNAc-T1, and 47.4 % identity to mNGalNAc-T2. The NGalNAc-T2 has 46.5 % identity to mNGalNAc-T1, and 82.6 % identity to mNGalNAc-T2. The mNGalNAc-T1 has 46.3 % identity to mNGalNAc-T2.

The NGalNAc-T1 has 26.1 % identity in 226 amino acids of C terminus to CSGalNAc-T1, while the NGalNAc-T2 has

21.6 % identity in 431 amino acids of C terminus to  
CSGalNAc-T1 and 25.0 % identity in 224 amino acids of C  
terminus to CSGalNAc-T2.

Further, the NGalNAc-T1 has 19.3 % identity to human  
5 chondroitin synthase 1 (hCSS1) and 18.0 % identity to mouse  
chondroitin synthase 1 (mCSS1), while the NGalNAc-T2 has  
18.2 % to hCSS1 and 18.1% to mCSS1.

The mNGalNAc-T1 has 18.5 % identity to hCSS1 and  
18.1 % identity to mCSS1, while the mNGalNAc-T2 has 18.1 %  
10 identity to hCSS1 and 18.8 % identity to mCSS1.

Therefore, it is recognized that the protein of the  
present invention is a novel one.

In addition, the protein of the present invention has  
the identity of 27 or more % to the amino acid sequence of  
15 SEQ ID NO: 1 or 3.

The protein of the present invention has the identity  
of 19 or more % to the amino acid sequence of SEQ ID NO: 26  
or 28.

In addition, GENETYX is a genetic information  
20 processing software for nucleic acid analysis and protein  
analysis, which is capable of performing general homology  
analysis and multiple alignment analysis, as well as  
calculating a signal peptide, a site of promoter, and  
secondary structure. The program for homology analysis used  
25 herein adopts the Lipman-Pearson method (Lipman, D. J. &  
Pearson, W. R., Science, 277, 1435-1441 (1985)) which is  
frequently used as a high speed, highly sensitive method.

The amino acid sequences of the proteins and the DNA  
sequences encoding them disclosed herein can be wholly or



partially used to readily isolate genes encoding proteins having a similar physiological activity from that of other species using genetic engineering techniques including hybridization and nucleic acid amplification reactions such as PCR. In such cases, novel proteins encoded by these genes can also be included in the scope of the present invention.

Proteins of the present invention may contain an attached sugar chain if they have an amino acid sequence as defined above as well as the enzymatic activity described above.

More specifically, as described in Examples 2 and 5 below, from the search of an acceptor substrate to the protein of the present invention, said protein acts to transfer GalNAc to GlcNAc via a  $\beta$ 1-4 linkage.

Furthermore specifically, the proteins of the present invention have at least one of the following properties (A)-(C), preferably all of these properties:

(A) Specificity of acceptor substrates

(a) When O-linked oligosaccharides are used as an acceptor substrate, said proteins have the activity of transferring N-acetylgalactosamine to GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$ -pNp (hereinafter, "core2-pNp"), GlcNAc $\beta$ 1-3GalNAc $\alpha$ -pNp (hereinafter, "core3-pNp"), GlcNAc $\beta$ 1-6GalNAc $\alpha$ -pNp (hereinafter, "core6-pNp") via a  $\beta$ 1-4 linkage, wherein the abbreviations used are: GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine; Gal, galactose; pNp, p-nitrophenyl. Preferably, said proteins have the transferring activity to core6-pNp.

(b) When N-linked oligosaccharides are used as an

acceptor substrate, said proteins have the activity of transferring N-acetylgalactosamine to GlcNAc at the non-reducing end of said oligosaccharides via a  $\beta$ 1-4 linkage, provided that said activity reduces when said

5 oligosaccharides have the following properties:

(i) having fucose (Fuc) residues in the structure of said oligosaccharides; and

(ii) having one or more branched chains wherein GalNAc residues bind to GlcNAc residues at the non-reducing end.

10 (B) Optimum pH in enzymatic activity

The activity tends to be higher in pH 6.5 of MES (2-morpholineethanesulfonic acid) buffer. In HEPES ([4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer, the activity tends to be higher in pH 6.75 for NGalNAc-T1 and pH

15 7.4 for NGalNAc-T2.

(C) Requirement of divalent ions

In NGalNAc-T1, the activity tends to be higher in the MES buffer including at least  $Mn^{2+}$ , or  $Co^{2+}$ , preferably  $Mn^{2+}$ . In NGalNAc-T2, the activity tends to be higher in the MES

20 buffer including  $Mg^{2+}$ ,  $Mn^{2+}$ , or  $Co^{2+}$ , preferably  $Mg^{2+}$ .

(2) Nucleic acids

Nucleic acids of the present invention include DNA in both single-stranded and double-stranded forms, as well as  
25 the RNA complements thereof. DNA includes, for example, native DNA, recombinant DNA, chemically synthesized DNA, DNA amplified by PCR and combinations thereof. The nucleic acid of the present invention is preferably a DNA.

The nucleic acids of the present invention are

nucleic acids (including the complement thereof) encoding the amino acids shown in SEQ ID NO: 1, 3, 26 or 28.

Typically, the nucleic acids of the present invention have the nucleic acid sequence of SEQ ID NO: 2, 4, 27 or 29

5 (including the complements thereof), which are clones obtained in the working example below which shows simply an example of the present invention. It is well-known for a person skilled in the art that in native nucleic acids, there are minor mutants derived from various kinds of  
10 species which produce them and ecotypes and mutants from a presence of isozymes. Therefore, the nucleic acids of the present invention include, but are not limited to, the nucleic acids having the nucleic acid sequence shown in SEQ ID NO: 2, 4, 27 or 29. The nucleic acids of the present  
15 invention include all nucleic acids encoding the proteins of the present invention.

Particularly, the amino acid sequences of the proteins and the DNA sequences encoding them disclosed herein can be wholly or partially used to readily isolate  
20 nucleic acids encoding proteins having a similar physiological activity from that of other species using genetic engineering techniques including hybridization and nucleic acid amplification reactions such as PCR. In such cases, such nucleic acids can also be included in the scope  
25 of the present invention.

As used herein, "stringent conditions" means hybridization under conditions of moderate or high stringency. Specifically, conditions of moderate stringency can be readily determined by those having ordinary skill in

the art based on, for example, the length of the DNA. The basic conditions are shown by Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd edition, Vol. 1, 7.42-7.45 Cold Spring Harbor Laboratory Press, 2001 and include use of  
5 a prewashing solution for the nitrocellulose filters of 5 × SSC, 0.5 % SDS, 1.0 mM EDTA (pH 8.0), hybridization conditions of about 50 % formamide, 2 × SSC - 6 × SSC at about 40-50 °C (or other similar hybridization solution such as Stark's solution, in about 50 % formamide at about  
10 42 °C), and washing conditions of 0.5 × SSC, 0.1 % SDS at about 60 °C. Conditions of high stringency can also be readily determined by those skilled in the art based on, for example, the length of the DNA. Generally, such conditions include hybridization and/or washing at a higher temperature  
15 and/or a lower salt concentration as compared with conditions of moderate stringency and are defined as hybridization conditions as above followed by washing in 0.2 × SSC, 0.1 % SDS at about 68 °C. Those skilled in the art will recognize that the temperature and the salt  
20 concentration of the washing solution can be adjusted as necessary according to factors such as the length of the probe.

Nucleic acid amplification reactions include reactions involving temperature cycles such as polymerase  
25 chain reaction (PCR) [Saiki R.K. et al., Science, 230, 1350-1354 (1985)], ligase chain reaction (LCR) [Wu D.Y. et al., Genomics, 4, 560-569 (1989); Barringer K.J. et al., Gene, 89, 117-122 (1990); Barany F., Proc. Natl. Acad. Sci. USA, 88, 189-193 (1991)] and transcription-based amplification [Kwoh

D.Y. et al., Proc. Natl. Acad. Sci. USA, 86, 1173-1177 (1989)] as well as isothermal reactions such as strand displacement amplification (SDA) [Walker G.T. et al., Proc. Natl. Acad. Sci. USA, 89, 392-396 (1992); Walker G.T. et al.,  
5 Nuc. Acids Res., 20, 1691-1696 (1992)], self-sustained sequence replication (3SR) [Guatelli J.C., Proc. Natl. Acad. Sci. USA, 87, 1874-1878 (1990)], and Q $\beta$  replicase system [Lizardi et al., BioTechnology, 6, 1197-1202 (1988)]. Other reactions such as nucleic acid sequence-based amplification  
10 (NASBA) using competitive amplification of a target nucleic acid and a variant sequence disclosed in European Patent No. 0525882 can also be used. PCR is preferred.

Homologous nucleic acids cloned by hybridization, nucleic acid amplification reactions or the like as  
15 described above have an identity of at least 50 % or more, preferably 60 % or more, more preferably 70 % or more, even more preferably 80 % or more, still more preferably 90 % or more, and most preferably 95 % or more to the nucleotide sequence of SEQ ID NO: 2, 4, 27 or 29 in the Sequence  
20 Listing.

The percentage identity of nucleic acid sequences may be determined by visual inspection and mathematical calculation. Alternatively, the percentage identity of two nucleic acid sequences can be determined by comparing  
25 sequence information using the GAP computer program, version 6.0 described by Devereux et al., Nucl. Acids Res., 12:387 (1984) which is available from the University of Wisconsin Genetics Computer Group (UWCGC). The preferred default parameters for the GAP program include: (1) a unary

comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, Nucl. Acids Res., 14:6745 (1986), as described by Schwartz and Dayhoff, eds; 5 Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by one skilled in the art of sequence 10 comparison may also be used.

When the identity search of the nucleic acid of the present invention is performed using GENETYX (Genetyx Co.), the NGalNAC-T1 has 59.7 % identity to NGalNAC-T2, 81.4 % identity to mNGalNAC-T1, and 59.0 % identity to mNGalNAC-T2. 15 The NGalNAC-T2 has 59.7 % identity to mNGalNAC-T1, and 83.4 % identity to mNGalNAC-T2. The mNGalNAC-T1 has 59.6 % identity to mNGalNAC-T2.

The NGalNAC-T1 has 44.6 % identity to hCSS1 and 46.0 % identity to mCSS1, while the NGalNAC-T2 has 47.3 % to 20 hCSS1 and 47.9 % to mCSS1.

The mNGalNAC-T1 has 46.4 % identity to hCSS1 and 46.6 % identity to mCSS1, while mNGalNAC-T2 has 48.6 % identity to hCSS1 and 48.7 % identity to mCSS1.

Therefore, it is recognized that the nucleic acid of 25 the present invention is a novel one.

In addition, the nucleic acid of the present invention has the identity of 48 or more % to the amino acid sequence of SEQ ID NO: 2 or 4.

The nucleic acid of the present invention has the

identity of 49 or more % to the amino acid sequence of SEQ  
ID NO: 27 or 29.

(3) Recombinant vectors and transformants

5           The present invention provides the recombinant  
vectors containing the nucleic acid of the present invention.  
Methods for integrating a DNA fragment of a nucleic acid of  
the present invention into a vector such as a plasmid are  
described in, for example, Sambrook, J. et al., Molecular  
10 Cloning, A Laboratory Manual (3rd edition), Cold Spring  
Harbor Laboratory, 1.1 (2001). Commercially available  
ligation kits (e.g., those available from Takara Shuzo Co.,  
Ltd.) can be conveniently used. Thus obtained recombinant  
vectors (e.g., recombinant plasmids) are introduced into  
15 host cells (e.g., E. coli, TB1, LE392, or XL-1Blue, etc.).

Suitable methods for introducing a plasmid into a  
host cell include the use of calcium chloride or calcium  
chloride/rubidium chloride or calcium phosphate,  
electroporation, electro injection, chemical treatment with  
20 PEG or the like, and the use of a gene gun as described in  
Sambrook, J. et al., Molecular Cloning, A Laboratory Manual  
(3rd edition), Cold Spring Harbor Laboratory, 16.1 (2001).

Vectors can be conveniently prepared by linking a  
desired gene by a standard method to a recombination vector  
25 available in the art (e.g., plasmid DNA). Specific examples  
of suitable vectors include, but are not limited to, E.  
coli-derived plasmids such as pBluescript, pUC18, pUC19 and  
pBR 322.

In order to produce desired proteins, especially,

expression vectors are useful. The types of expression vectors are not specifically limited to those having the ability to express a desired gene in various prokaryotic and/or eukaryotic host cells to produce a desired protein, but preferably include expression vectors for *E. coli* such as pQE-30, pQE-60, pMAL-C2, pMAL-p2, pSE420; expression vectors for yeasts such as pYES2 (genus *Saccharomyces*), pIC3.5K, pPIC9K, pA0815 (all belonging to genus *Pichia*); and expression vectors for insects such as pBacPAK8/9, pBK283, pVL1392, pBlueBac4.5.

A transformant can be produced by introducing a desired expression vector into a host cell. The host cells employed are not specifically limited to those having the ability to be compatible to the expression vector of the present invention and to be able to be transformed, but various kinds of cells such as native cells are usually used in the art or recombinant cells are artificially established. For example, bacteria (genus *Escherichia*, genus *Bacillus*), yeasts (genus *Saccharomyces*, genus *Pichia*, etc.), mammalian cells, insect cells, and plant cells are exemplified.

The host cells are preferably *E. coli*, yeasts and insect cells, which are exemplified as *E. coli* (M15, JM109, BL21, etc.), yeasts (INVSc1 (genus *Saccharomyces*), GS115, KM71 (genus *Pichia*), etc.), and insect cells (BmN4, bombic larva, etc.). Examples of animal cells are mouse, *Xenopus*, rat, hamster, monkey or human derived cells or culture cell lines established from these cells. More specifically, the host cell is preferably COS cell which is a cell line derived from a kidney of monkey.



When a bacterium, especially E. coli is used as a host cell, the expression vector typically consists of at least a promoter/operator region, a start codon, a gene encoding a desired protein, a stop codon, a terminator and a replicable unit.

When a yeast, plant cell, animal cell or insect cell is used as a host cell, the expression vector typically preferably contains at least a promoter, a start codon, a gene encoding a desired protein, a stop codon and a terminator. It may also contain a DNA encoding a signal peptide, an enhancer sequence, untranslated regions at the 5' and 3' ends of a desired gene, a selectable marker region or a replicable unit, etc., if desired.

Preferred start codons in vectors of the present invention include a methionine codon (ATG). Stop codons include commonly used stop codons (e.g., TAG, TGA, TAA).

The replicable unit means DNA capable of replicating the entire DNA sequence in a host cell, such as natural plasmids, artificially modified plasmids (plasmids prepared from natural plasmids), synthetic plasmids, etc. Preferred plasmids include plasmid pQE30, pET or pCAL or their artificial variants (DNA fragments obtained by treating pQE30, pET or pCAL with suitable restriction endonucleases) for E. coli; plasmid pYES2 or pPIC9K for yeasts; and plasmid pBacPAK8/9 for insect cells.

Enhancer sequences and terminator sequences may be those commonly used by those skilled in the art such as those derived from SV40.

As for selectable markers, those commonly used can be

used by standard methods. Examples are genes resistant to antibiotics such as tetracycline, ampicillin, kanamycin, neomycin, hygromycin or spectinomycin.

Expression vectors can be prepared by linking at least a promoter, a start codon, a gene encoding a desired protein, a stop codon and a terminator region as described above to a suitable replicable unit in series into a circle. While carrying out the linking process, a suitable DNA fragment (such as a linker or another restriction site) can be used by standard methods such as digestion with a restriction endonuclease or ligation with T4 DNA ligase, if desired.

Introduction [transformation (transduction)] of expression vectors of the present invention into host cells can be performed by using known techniques.

For example, bacteria (such as *E. coli*, *Bacillus subtilis*) can be transformed by the method of Cohen et al. [Proc. Natl. Acad. Sci. USA, 69, 2110 (1972)], the protoplast method [Mol. Gen. Genet., 168, 111 (1979)] or the competent method [J. Mol. Biol., 56, 209 (1971)]; *Saccharomyces cerevisiae* can be transformed by the method of Hinnen et al [Proc. Natl. Acad. Sci. USA, 75, 1927 (1978)] or the lithium method [J.B. Bacteriol., 153, 163 (1983)]; plant cells can be transformed by the leaf disc method [Science, 227, 129 (1985)] or electroporation [Nature, 319, 791 (1986)]; animal cells can be transformed by the method of Graham [Virology, 52, 456 (1973)]; and insect cells can be transformed by the method of Summers et al. [Mol. Cell. Biol., 3, 2156-2165 (1983)].

#### (4) Isolation/purification of proteins

Proteins of the present invention can be expressed (produced) by culturing transformed cells containing an expression vector prepared as described above in a nutrient medium. The nutrient medium preferably contains a carbon, inorganic nitrogen or organic nitrogen source necessary for the growth of host cells (transformants). Examples of carbon sources include glucose, dextran, soluble starch, sucrose and methanol. Examples of inorganic or organic nitrogen sources include ammonium salts, nitrates, amino acids, corn steep liquor, peptone, casein, beef extract, soybean meal and potato extract. If desired, other nutrients (e.g., inorganic salts such as sodium chloride, calcium chloride, sodium dihydrogen phosphate and magnesium chloride; vitamins; antibiotics such as tetracycline, neomycin, ampicillin and kanamycin) may be contained. Incubation of cultures takes place by techniques known in the art. Culture conditions such as temperature, the pH of the medium and the incubation period are appropriately selected to produce a protein of the present invention in mass.

Proteins of the present invention can be obtained from the resulting cultures as follows. That is, when proteins of the present invention accumulate in host cells, the host cells are collected by centrifugation or filtration or the like and suspended in a suitable buffer (e.g., a buffer such as a Tris buffer, a phosphate buffer, an HEPES buffer or an MES buffer at a concentration of about 10 M -

100 mM desirably at a pH in the range of 5.0 - 9.0, though the pH depends on the buffer used), then the cells are disrupted by a method suitable for the host cells used and centrifuged to collect the contents of the host cells. When  
5 proteins of the present invention are secreted from host cells, the host cells and culture medium are separated by centrifugation or filtration or the like to give a culture filtrate. The disruption solution of the host cells or the culture filtrate can be used to isolate/purify a protein of  
10 the present invention directly or after ammonium sulfate precipitation and dialysis. An isolation/purification method is as follows. When the protein of interest is tagged with 6 x histidine, GST, maltose-binding protein or the like, conventional methods based on affinity chromatography  
15 suitable for each tag can be used. When the protein of the present invention is produced without using these tags, the method described in detail in the examples below based on ion exchange chromatography can be used, for example. These methods may be combined with gel filtration chromatography,  
20 hydrophobic chromatography, isoelectric chromatography or the like.

N-acetylgalactosamine is transferred by the action of proteins of the present invention on glycoprotein, oligosaccharide, polysaccharide or the like having N-  
25 acetylglucosamine. Thus, proteins of the present invention can be used to modify a sugar chain of a glycoprotein or to synthesize a sugar. Moreover, the proteins can be administered as immunogens to an animal to prepare antibodies against said proteins, and said antibodies can be

used to determine said proteins by immunoassays. Thus, proteins of the present invention and the nucleic acids encoding them are useful in the preparation of such immunogens.

5           Further, proteins of the present invention can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in US Patent No. 5,011,912 and in Hopp et al., Bio/Technology, 10 6:1204, 1988. One such peptide is the FLAG® peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQ ID NO: 30) which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A 15 murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG® peptide in the presence of certain divalent metal cations, as described in US Patent No. 5,011,912 hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American 20 Type Culture Collection under Accession No. HB 9259. Monoclonal antibodies that bind the FLAG® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

          Specifically, the cDNA of the FLAG is inserted into 25 an expression vector expressing a protein of the present invention to express the FLAG-tagged protein, after which the expression of the protein of the present invention can be confirmed by an anti-FLAG antibody.

(5) Analytical nucleic acid

According to the present invention, a nucleic acid which hybridizes to the nucleic acids of the present invention (hereinafter referred to as "analytical nucleic acid") is provided. The analytical nucleic acid of the present invention includes, but is not limited to, typically, native or synthesized fragments derived from nucleic acid encoding the protein of the present invention. As used herein, the term "analytical" includes any of detection, amplification, quantitative and semi-quantitative assays.

(a) Primers

When analytical nucleic acids of the present invention are used as primers for nucleic acid amplification reactions, the analytical nucleic acids of the present invention are oligonucleotides prepared by a process comprising:

selecting two regions from the nucleotide sequence of a gene encoding a protein of SEQ ID NO: 1, 3, 26 or 28 to satisfy the conditions that:

1) each region should have a length of 15-50 bases;  
and

2) the proportion of G + C in each region should be 40-70 %;

generating a single-stranded DNA having a nucleotide sequence identical to or complementary to that of said region or generating a mixture of single-stranded DNAs taking into account degeneracy of the genetic code so that the amino acid residue encoded by said single-stranded DNA

is retained, and, as necessary, generating the single-stranded DNA containing a modification without affecting the binding specificity to the nucleotide sequence of the gene encoding said protein.

5           Primers of the present invention preferably have a sequence homologous to that of a partial region of a nucleic acid of the present invention, but one to two bases may be mismatched.

10           Primers of the present invention contain 15 bases or more, preferably 18 bases or more, more preferably 21 bases or more, and 50 bases or fewer bases.

15           The primer of the present invention has typically the nucleic acid sequence selected of a group consisting of SEQ ID NO: 20, 21, 23 and 24, and can be used as a single primer or a suitably combined pair of primers. These nucleotide sequences were designed based on amino acid sequence of SEQ ID 1 or 3 as a PCR primer for cloning gene fragments encoding each protein. The sequence is a primer mixed with all nucleic acids capable of encoding said amino acids.

20

#### (b) Probes

25           When analytical nucleic acids of the present invention are used as probes, the analytical nucleic acids of the present invention preferably have a sequence homologous to that of a total or partial region of the nucleotide sequence of SEQ ID NO: 2, 4, 27 or 29, and further, may have a mismatch of one or two bases. The probes of the present invention have a length of 15 bases and more, preferably 20 bases and more, and within a full

length of the encoding region, that is, 3120 bases  
(corresponding to SEQ ID NO: 2), 2997 bases (corresponding  
to SEQ ID NO: 4), 3105 bases (corresponding to SEQ ID NO:  
27), or 2961 bases (corresponding to SEQ ID NO: 29). The  
5 probes have typically the nucleic acid sequence shown in SEQ  
ID NO: 22 or 25. The probes may be obtained from native  
nucleic acid treated with restriction enzymes, or may be  
synthesized oligonucleotides.

Probes of the present invention include labeled  
10 probes having a label such as a fluorescent, radioactive or  
biotinylation label to detect or confirm that the probes  
have hybridized to a target sequence. The presence of a  
nucleic acid to be tested in an analyte can be determined by  
immobilizing the nucleic acid to be tested or an  
15 amplification product thereof, hybridizing it to a labeled  
probe, and after washing, measuring the label bound to the  
solid phase. Alternatively, it can also be determined by  
immobilizing the analytical nucleic acid, hybridizing to the  
nucleic acid to be tested and detecting the nucleic acid to  
20 be tested coupled to the solid phase with a labeled probe or  
the like. In the latter case, the immobilized analytical  
nucleic is also referred to as a probe.

Generally, nucleic acid amplification methods such as  
PCR can be readily performed because they are per se well  
25 known in the art, and reagent kits and apparatus for them  
are also commercially available. When a nucleic acid  
amplification method is performed using a pair of analytical  
nucleic acids of the present invention described above as  
primers and a nucleic acid to be tested as the template, the



presence of the nucleic acid to be tested in a sample can be known by detecting an amplification product because the nucleic acid to be tested is amplified while no amplification occurs when the nucleic acid to be tested is not contained in the sample. The amplification product can be detected by electrophoresing the reaction solution after amplification, staining the bands with ethidium bromide, immobilizing the amplification product after electrophoresis to a solid phase such as a nylon membrane, hybridizing the immobilized product with a labeled probe that specifically hybridizes to the nucleic acid to be tested, and washing the hybridization product and then detecting said label. Further, the amount of the nucleic acid to be tested in a sample can also be determined by the so-called real-time PCR detection using a quencher fluorescent dye and a reporter fluorescent dye. This method can also be readily carried out using a commercially available real-time PCR detection kit. The nucleic acid to be tested can also be semi-quantitatively assayed based on the intensity of electrophoretic bands. The nucleic acid to be tested may be mRNA or cDNA reversely transcribed from mRNA. When mRNA is to be amplified as the nucleic acid to be tested, the NASBA methods (3SR, TMA) can also be adopted using said pair of primers. The NASBA methods can be readily performed because they are per se well known and kits for them are commercially available.

#### (c) Microarrays

Analytical nucleic acids of the present invention can

be used as microarrays. Microarrays are means for enabling rapid large-scale data analysis of genomic functions. Specifically, a labeled nucleic acid is hybridized to a number of different nucleic acid probes immobilized in high  
5 density on a solid substrate such as a glass substrate, a signal from each probe is detected and the collected data are analyzed. As used herein, the "microarray" means an array of an analytical nucleic acid of the present invention on a solid substrate such as a membrane, filter, chip or  
10 glass surface.

#### (6) Antibodies

An antibody that is immunoreactive with the protein of the present invention is provided herein. Such an  
15 antibody specifically binds to the polypeptide via the antigen-binding site of the antibody (as opposed to non-specific binding). Therefore, as set forth above, proteins of SEQ ID NOs: 1 and 3, fragments, variants, and fusion proteins and the like can be used as "immunogens" in  
20 producing antibodies immunoreactive therewith. More specifically, the proteins, fragments, variants, and fusion proteins and the like include the antigenic determinants or epitopes to induce the formation of an antibody. Such antigenic determinants or epitopes may be either linear or  
25 conformational (discontinuous). In addition, said antigenic determinants or epitopes may be identified by any methods known in the art.

Therefore, one aspect of the present invention relates to the antigenic epitopes of the protein of the

present invention. Such epitopes are useful raising antibodies, in particular monoclonal antibodies, as described in more detailed below. Additionally, epitopes from the protein of the present invention can be used as research reagents, in assays, to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a protein, or by using recombinant DNA technology.

As for antibodies which can be induced by the proteins of the present invention, both polyclonal and monoclonal antibodies can be prepared by conventional techniques, whether a whole body or a part of said proteins have been isolated, or the epitopes have been isolated. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, NY, 1980.

Hybridoma cell lines that produce monoclonal antibodies specific for the proteins of the present invention are also contemplated herein. Such hybridomas can be produced and identified by conventional techniques. One method for producing such a hybridoma cell line comprises immunizing an animal with a protein of the present invention; harvesting spleen cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds said protein. The monoclonal antibodies can be

recovered by conventional techniques.

The antibodies of the present invention include chimeric antibodies such as humanized versions of murine monoclonal antibodies. Such humanized antibodies can be prepared by known techniques and offer the advantages of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen-binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment can comprise the antigen-binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody.

The present invention includes antigen-binding antibody fragments that can be also generated by conventional techniques. Such fragments include, but are not limited to, Fab and  $F(ab')_2$  as an example. Antibody fragments generated by genetic engineering techniques and derivatives thereof are also provided.

In one embodiment, the antibody is specific to the protein of the present invention, and it does not cross-react with other proteins. Screening procedures by which such antibodies can be identified are publicly known, and may involve, for example, immunoaffinity chromatography.

The antibodies of the invention can be used in assays to detect the presence of the protein or fragments of the present invention, either in vitro or in vivo. The antibodies also can be used in purifying proteins or

fragments of the present invention by immunoaffinity chromatography.

Further, a binding partner such as an antibody that can block binding of a protein of the present invention to an acceptor substrate can be used to inhibit a biological activity rising from such a binding. Such a blocking antibody may be identified by any suitable assay procedure, such as by testing the antibody for the ability to inhibit binding of said protein to specific cells expressing the acceptor substrate. Alternatively, a blocking antibody can be identified in assays for the ability to inhibit a biological effect that results from a protein of the present invention binding to the binding partner of target cells.

Such an antibody can be used in an in vitro procedure, or administered in vivo to inhibit a biological activity mediated by the entity that generated the antibody.

Disorders caused or exacerbated (directly or indirectly) by the interaction of a protein of the present invention with a binding partner thus can be treated. A therapeutic method involves in vivo administration of a blocking antibody to a mammal in an amount effective to inhibit a binding partner-mediated biological activity. Monoclonal antibodies are generally preferred for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is used.

#### (7) Cancer markers and methods for detection

The protein or nucleic acids of the present invention can be used as a cancer marker, and be applied to diagnosis and treatment of cancers and the like. As used herein, the

term "cancer" means typically all malignant tumors, and includes disease conditions with said malignant tumors. "Cancer" includes, but is not limited to, lung cancer, liver cancer, kidney cancer and leukemia.

5 "Cancer marker" used herein means the protein and nucleic acids of the present invention that express more than those of a non-cancerous biological sample, when a biological sample is cancerous. In addition, "biological sample" includes tissues, organs, and cells. Blood is  
10 preferable, pathological tissue is more preferable.

Specifically, when the protein of the present invention is used as a cancer marker, a method for detection of the present invention includes the steps: (a) quantifying said protein in a biological sample; and (b) estimating that  
15 the biological sample is cancerous in the case that the quantity value of said protein in the biological sample is more than that in a control biological sample. In said method for detection, the antibody of the present invention can be used to quantify said protein of the biological  
20 sample. According to the present invention, generally, the method for qualifying the protein is not limited to the above methods and can use quantity methods know in the art such as ELISA, Western Blotting. A ratio of the quantity value is preferably 1.5 times or more, more preferably 3  
25 times or more, and even more preferably 10 times or more.

On the other hand, when the nucleic acid of the present invention is used as a cancer marker, a method for detection of the present invention includes the steps of: (a) quantifying said nucleic acid in a biological sample;

and (b) estimating that the biological sample is cancerous in the case that the quantity value of said nucleic acid in the biological sample is 1.5 times or more than that of a control biological sample. Preferably, the steps comprise

5 (a) hybridizing at least one of said analytical nucleic acids to said nucleic acid in the biological sample; (b) amplifying said nucleic acid; (c) hybridizing said nucleic acids to the amplification product; (d) quantifying a signal rising from said amplification product and said analytical

10 nucleic acid hybridized; and (e) estimating that the biological sample is cancerous in the case that the quantity value of said signal is 1.5 times or more than that of a corresponding signal of a control biological sample.

More specifically, as described in the example below,

15 canceration can be estimated by determination of a ratio of expression level of the nucleic acids in cancerous tissue and normal tissue by quantitative PCR. According to the present invention, the quantification of the nucleic acid is not limited to this, and for example, RT-PCR, northern

20 blotting, dot blotting or DNA microarray may be used. In such quantification, nucleic acids of genes present generally and broadly in same tissue and the like such as nucleic acids encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH),  $\beta$ -actin are used as a control. A

25 quantity ratio to be estimated as canceration is preferably 1.5 or more, more preferably 3 or more, even more preferably 10 or more.

The following examples further illustrate the present

invention without, however, limiting the invention thereto.

### Examples

5 Example 1 Preparation of the human protein of the present invention

1. Search through a genetic database and determination of the nucleic acid sequence of a novel N-acetylgalactosamine  
10 transferase

A search of similar genes through a genetic database was performed by use of the genes for existing  $\beta$ -1,4-galactose transferases. The sequences used were SEQ ID NOs: AL161445, AF038660, AF038661, AF022367, AF038663, AF038664  
15 in the genes for  $\beta$ -1,4-galactose transferases. The search was performed using a program such as Blast [Altschul et al., J. Mol. Biol., 215, 403-410 (1990)].

As a result, GenBank Accession No. N48738 was found as an EST sequence, and GenBank Accession No. AC006205 was  
20 found as a genome sequence. As a further result, it is considered that both sequences comprise disparate genes (hereinafter, the genes comprising N48738 and AC006205 refer to NGalNAc-T1 and NGalNAc-T2, respectively). Since the translation initiation sites of both genes were unknown, it  
25 was impossible to predict the full length of the genes. Marathon-Ready cDNA (Human Brain or Stomach) from CLONTECH was used for obtaining the information of coding regions (5' RACE: Rapid Amplification of cDNA Ends) and cloning.



Obtaining information of coding region of NGalNAc-T1

AP1 primer included in Marathon cDNA (a DNA fragment having adaptors AP1 and AP2 at both ends) and primer K12R6 generated within the identified sequence part (5'-GCT CCT GCA GCT CCA GCT CCA-3') (SEQ ID NO: 5) were used for PCR (30 cycles of 94 °C for 20 seconds, 60 °C for 30 seconds and 72 °C for 2 minutes). Further, AP2 primer included in Marathon cDNA and primer K12R5 generated within the identified sequence part (5'-AAG CGA CTC CCT CGC GCC GAG T-3') (SEQ ID NO: 6) were used for nested PCR (30 cycles of 94 °C for 20 seconds, 60 °C for 30 seconds and 72 °C for 2 minutes). A fragment of about 0.6 kb obtained as a result was purified by a common method, and the nucleic acid sequence was analyzed. However, since a transmembrane sequence special to glycosyl transferases (hydrophobic 20 amino acids) could have appeared, an EST sequence (GenBank Accession No. PF058197) was discovered based on the obtained sequence and the nucleic acid sequence of NGalNAc-T2 described later by search through genome database. Based on the information of nucleic acid sequence, RT-PCR was performed using two primers (K12F101: 5'-ATG CCG CGG CTC CCG GTG AAG AAG-3' (SEQ ID NO: 7) and K12R5) and the amplification was confirmed. Therefore, it was explained that this EST sequence and the sequence obtained by 5' RACE exist on one mRNA. The full length of nucleotide sequence (3120 bp) was shown in SEQ ID NO: 2.

Obtaining information of coding region of NGalNAc-T2

AP1 primer included in Marathon cDNA (a DNA fragment

having adaptors AP1 and AP2 at both ends) and primer K13-R3 generated within the identified sequence part (5'-CAA CAG TTC AAG CTC CAG GAG GTA-3' (SEQ ID NO: 8)) were used for PCR (30 cycles of 94 °C for 20 seconds, 60 °C for 30 seconds and 72 °C for 2 minutes). Further, AP2 primer included in Marathon cDNA and primer K13R2 generated within the identified sequence part (5'-CTG ACG CTT TTC CAC GTT CAC AAT-3' (SEQ ID NO: 9)) were used for nested PCR (30 cycles of 94 °C for 20 seconds, 60 °C for 30 seconds and 72 °C for 2 minutes). A fragment of about 1.0 kb obtained as a result was purified by a common method, and the nucleic acid sequence was analyzed. Further, a coding region of a protein was determined. However, since a transmembrane sequence special to glycosyl transferases (hydrophobic 20 amino acids) could have appeared, further 3 times 5' RACE was performed. The primers used here are shown in Table 2.

As a result, the obtained full length of nucleotide sequence (2997 bp) was shown in SEQ ID NO: 4.

Table 2 Various primers used in RACE

Second 5' RACE primers

K13 R6 5'-CAC CCC GTC TCT GCT CTG CGA T-3' (SEQ ID NO: 10)

K13 R5 5'-GTC TTC CTG GGG CTG TCA CCA-3' (SEQ ID NO: 11)

Third 5' RACE primers

K13 R7 5'-CAC CTC ATC CAT CTG TAG GAA CGT-3' (SEQ ID NO: 12)

K13 R8 5'-CTG TCG CCA TGC AAC TTC CAC GT-3' (SEQ ID NO: 13)

Fourth 5' RACE primers

K13 R12 5'-AAT GTC GTG GTC CTC GAG GCT CA-3' (SEQ ID NO: 14)

K13 R11 5'-GAT GGT AGA ACT GGA GGT GTG GAT-3' (SEQ ID NO: 15)

## 2. Integration of GalNAc-T gene into an expression vector

To prepare an expression system of GalNAc-T, a  
5 portion of GalNAc-T gene was first integrated into pFLAG-CMV1 (Sigma).

### Integration of NGalNAc-T1 into pFLAG-CMV1

A region corresponding to amino acids 62-1039 of SEQ  
10 ID NO: 1 or 2 was amplified by LA Taq DNA polymerase (Takara Shuzo) using Marathon cDNA (Human Brain) as a template, forward primer K12-Hin-F2: 5'-CCC AAG CTT CGG GGG GTC CAC GCT GCG CCA T-3' (SEQ ID NO: 16), and reverse primer K12-Xba-R1: 5'-GCT CTA GAC TCA AGA CGC CCC CGT GCG AGA-3' (SEQ  
15 ID NO: 17). The fragment was digested at restriction sites (HindIII and XbaI) included in the primers, and inserted into pFLAG-CMV1 digested with Hind III and XbaI by use of Ligation High (Toyobo) to prepare pFLAG-NGalNAc-T1.

### 20 Integration of NGalNAc-T2 into pFLAG-CMV1

A region corresponding to amino acids 57-998 of SEQ  
ID NO: 3 or 4 was amplified by LA Taq DNA polymerase (Takara Shuzo) using Marathon cDNA (Human Stomach) as a template, forward primer K13-Eco-F1: 5'-GGA ATT CGA GGT ACG GCA GCT  
25 GGA GAG AA-3' (SEQ ID NO: 18), and reverse primer K13-Sal-R1: 5'-ACG CGT CGA CCT ACA GCG TCT TCA TCT GGC GA-3' (SEQ ID NO: 19). This fragment was digested at restriction sites (EcoRI and SalI) included in the primers, and inserted temporally into pcDNA3.1 digested with EcoRI and SalI. This

was digested with EcoRI and PmeI. The fragment including the active site of NGalNAc-T2 was inserted at the EcoRI-EcoRV site of pFLAG-CMV1 using Ligation High (Toyobo Co.) to prepare pFLAG-NGalNAc-T2.

5

### 3. Transfection and expression of recombinant enzymes

15 µg of pFLAG-NGalNAc-T1 or pFLAG-NGalNAc-T2 was induced into  $2 \times 10^6$  of COS-1 cells which were cultured overnight in DMEM (Dulbecco's modified Eagle's medium) including 10 % FCS (fetal calf serum), using Lipofectamine 2000 (Invitrogen Co.) as a protocol provided by the same company. A supernatant of 48-72 hours was collected. The supernatant was mixed with  $\text{NaN}_3$  (0.05 %), NaCl (150 mM),  $\text{CaCl}_2$  (2 mM) and an anti-M1 resin (Sigma Co.) (50 µl), and the mixture was stirred overnight at 4 °C. The solution of reaction mixture was centrifuged (3000 rpm, 5 min, 4 °C) to collect a pellet. The pellet was combined with 900 µl of 2 mM  $\text{CaCl}_2$ /TBS and re-centrifuged (2000 rpm, 5 min, 4 °C), after which the pellet was suspended in 200 µl of 1 mM  $\text{CaCl}_2$ /TBS to give a sample for assaying activity (NGalNAc-T1 or NGalNAc-T2 enzyme solution).

The enzyme was subjected to conventional SDS-PAGE and Western blotting, and the expression of the intended protein was confirmed. Anti FLAG M2-peroxydase (A-8592, SIGMA Co.) was used as an antibody.

Example 2 Assay of activity using the enzyme of the present invention

# 1. Search for donor substrates

A search for a donor substrate of the enzyme of the present invention was performed on various mono-saccharide acceptor substrates, using 5 ml of enzyme solution and

5 various acceptor substrates.

The acceptor substrates were prepared so that each of Gal- $\alpha$ -pNp, Gal- $\beta$ -oNp, GalNAc- $\alpha$ -Bz, GalNAc- $\beta$ -pNp, GlcNAc- $\alpha$ -pNp, GlcNAc- $\beta$ -pNp, Glc- $\alpha$ -pNp, Glc- $\beta$ -pNp, GlcA- $\beta$ -pNp, Fuc- $\alpha$ -pNp, Man- $\alpha$ -pNp (thereinbefore, CALBIOCHEM Co.), Xyl- $\alpha$ -pNp, 10 Xyl- $\beta$ -pNp (thereinbefore, SIGMA Co.) was included in 2.5 nmol/20  $\mu$ l. Further, the solutions of various donor substrates (UDP-GalNAc, UDP-GlcNAc, UDP-Gal, GDP-Man, UDP-GlcA, UDP-Xyl and GDP-Fuc, thereinbefore, SIGMA Co.) are shown in Table 3.

15

Table 3

GalNAc-T		GlcA-T	
MES or HEPES (pH 5.5 -	50 mM	MES (pH 7.0)	50 mM
UDP-GalNAc	0.5 mM	UDP-GlcA	0.25 mM
UDP-[14C]GalNAc	2 nCi/ul	UDP-[14C]GlcA	2 nCi/ul
MnCl <sub>2</sub>	20 mM	MnCl <sub>2</sub>	10 mM
Tiron X-100	0.5%		
GlcNAc-T		Xyl-T	
HEPES (pH 7.0 or 7.5)	14 mM	MES (pH 7.0)	50 mM
UDP-GlcNAc	0.5 mM	UDP-Xyl	0.25 mM
UDP-[14C]GlcNAc	2 nCi/ul	UDP-[14C]Xyl	1 nCi/ul
MnCl <sub>2</sub>	10 mM	MnCl <sub>2</sub>	10 mM
Tiron CF-54	0.5%		
ATP	0.75 mM		
Gal-T		Fuc-T	
HEPES (pH 7.0 or 7.5)	14 mM	cacodylate buffer (pH 7.0)	50 mM
UDP-Gal	0.25 mM	GDP-[14C]Fuc	1 nCi/ul
UDP-[14C]Gal	2.5 nCi/ul	MnCl <sub>2</sub>	10 mM
MnCl <sub>2</sub>	10 mM	ATP	5 mM
ATP	0.75 mM		
		Man-T	
		Tris (pH 7.2)	50 mM
		GDP-[14C]Man	2 nCi/ul
		MnCl <sub>2</sub>	10 mM
		Triton X-100	0.6%

All of reaction times were 16 hours. After reaction, non-reactive acceptor substrates with radioactivity were removed with SepPack C18 column (Waters CO.), and radioactivity from donor substrates integrated into acceptor substrates was determined with a liquid scintillation counter. Consequently, there appeared little background even in UDP-GlcA using each of NGalNAc-T1 and NGalNAc-T2, however, the highest activity was detected in the case of UDP-GalNAc as a donor substrate.

10

## 2. Search for acceptor substrates

Further, in order to investigate acceptors, reactions were performed using each acceptor (10 nmol/20  $\mu$ l) by itself. As a result, significant radioactivity was detected in the case of GlcNAc- $\beta$ -pNp (NGalNAc-T1: 256.26 dpm, NGalNAc-T2: 1221.22 dpm). Based on the above results, it was explained that both of NGalNAc-T1 and NGalNAc-T2 are glycosyl transferases capable of transferring GalNAc to GlcNAc-T.

15

## 3. Study of optimum pH

As described above, it was explained that NGalNAc-T1 and NGalNAc-T2 are glycosyl transferases which transfer GalNAc to GlcNAc. Thereat, the optimum pH of both enzymes was studied. The buffer solutions used are MES (pH 5.5, 6.0, 6.26, 6.5, 6.75), HEPES (pH 6.75, 7.0, 7.4). As a result, as shown in Table 4, the activity tends to be higher in pH 6.5 of MES buffer for both NGalNAc-T1 and NGalNAc-T2.

25

Table 4 A result of optimum pH in enzymatic activity of  
NGalNAc-T1 and NGalNAc-T2

NGalNAc-T1

pH	Incorporation of radioactivity (A)	Blank (B)	(A) - (B)
MES buffer (pH 5.5)	339.76	263.21	76.55
MES buffer (pH 6.0)	321.04	263.21	57.83
MES buffer (pH 6.26)	636.34	263.21	373.13
MES buffer (pH 6.5)	1767.72	263.21	1504.51
MES buffer (pH 6.75)	923.92	263.21	660.71
HEPES buffer (pH 6.75)	1685.06	263.21	1421.85
HEPES buffer (pH 7.0)	1138.38	263.21	875.17
HEPES buffer (pH 7.4)	2587.48	263.21	2324.27

(dpm)

5

NGalNAc-T2

pH	Incorporation of radioactivity (A)	Blank (B)	(A) - (B)
MES buffer (pH 5.5)	336.20	263.21	72.99
MES buffer (pH 6.0)	341.92	263.21	78.71
MES buffer (pH 6.26)	339.50	263.21	76.29
MES buffer (pH 6.5)	753.62	263.21	490.05
MES buffer (pH 6.75)	529.24	263.21	266.03
HEPES buffer (pH 6.75)	915.16	263.21	651.95
HEPES buffer (pH 7.0)	786.70	263.21	523.49
HEPES buffer (pH 7.4)	586.32	263.21	323.11

(dpm)

In addition, the value (263.21 dpm) of MES (pH 6.75) was adopted as a blank value in the case of a non-enzyme.

10 Further, when pH of HEPES buffer was 7.4 for NGalNAc-T1 and 6.75 for NGalNAc-T2, the highest value was shown. However, the activity did not always increase even when pH increase.

Hereinafter, MES (pH 6.5) was used in each of experiments.

#### 4. Studying requirements of divalent cations

Generally, glycosyl transferases require frequently  
 5 divalent cations. The activity of each enzyme was studied  
 by adding various divalent cations. Consequently, the high  
 values were represented when  $Mn^{2+}$  in NGalNAc-T1, and  $Mg^{2+}$ ,  
 $Mn^{2+}$  and  $Co^{2+}$  in NGalNAc-T2 were added (see Table 5).  
 Regarding this, both enzymes showed the activity due to  
 10 adding EDTA which is a chelating agent. From the above  
 results, it was explained that both enzymes require divalent  
 cations.

15 Table 5 A result of requirements of divalent cations in the  
 activity of NGalNAc-T1 and NGalNAc-T2

##### NGalNAc-T1

Divalent cations etc.	Incorporation of radioactivity (A)	Blank (B)	(A) - (B)
MnCl <sub>2</sub>	519.47	263.21	256.26
MgCl <sub>2</sub>	256.36	263.21	-6.85
ZnCl <sub>2</sub>	210.29	263.21	-52.92
CaCl <sub>2</sub>	230.78	263.21	-32.43
CuCl <sub>2</sub>	278.77	263.21	15.56
CoCl <sub>2</sub>	240.91	263.21	-22.30
CdSO <sub>4</sub>	203.39	263.21	-59.82
EDTA	242.38	263.21	-20.83

(dpm)



## NGalNAc-T2

pH	Incorporation of radioactivity (A)	Blank (B)	(A) - (B)
MnCl <sub>2</sub>	1484.43	263.21	1221.22
MgCl <sub>2</sub>	3124.16	263.21	2860.95
ZnCl <sub>2</sub>	187.59	263.21	-75.62
CaCl <sub>2</sub>	217.83	263.21	-45.38
CuCl <sub>2</sub>	218.35	263.21	-44.86
CoCl <sub>2</sub>	1130.63	263.21	867.42
CdSO <sub>4</sub>	217.92	263.21	-45.29
EDTA	235.28	263.21	-27.93

(dpm)

Example 3 Expression analysis in various human tissues

5

The expression levels of said gene was quantified by quantitative PCR using cDNA of normal human tissues. The cDNA of normal tissues which was reversely transcribed from total RNA (CLONETECH Co.) was used. As for cell lines,

10 total RNA therefrom was extracted, and cDNA was prepared by conventional methods and was used. The quantitative expression analysis of NGalNAc-T1 was performed using primers: K12-F3 (5'-ctg gtg gat ttc gag agc ga-3' (SEQ ID NO: 20)) and K12-R3 (5'-tgc cgt cca gga tgt tgg-3' (SEQ ID

15 NO: 21)), and probe: K12-MGB3 (5'-gcg gta gag gac gcc-3' (SEQ ID NO: 22)). The quantitative expression analysis of NGalNAc-T2 was performed using primers: K13-F3 (5'-atc gtc atc act gac tat agc agt ga-3' (SEQ ID NO: 23)) and K13-R3 (5'-gaa tgg cat cga tga ctc cag-3' (SEQ ID NO: 24)), and

20 probe: K13-MGB3 (5'-ctc gtg aag gac ccg ca-3' (SEQ ID NO:

25)). A probe with a minor groove binder (Applied Biosystems Co.) was used. Universal PCR Master Mix was used as enzyme and reaction solution, and 25 ml of the reaction solution was quantified with ABI PRISM 7700 Sequence  
5 Detection System (together, Applied Biosystems Co.).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a standard gene for quantification. A calibration curve for quantification was made by using a template DNA at a known concentration, and the expression level of said gene was

10 normalized. Further, pFLAG-NGalNac-T1 and pFLAG-NGalNac-T2 were used as standard DNAs of NGalNac-T1 and NGalNac-T2.

The reaction temperature was 50 °C for 2 min, 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 sec, 60 °C for 1 min. The result is shown in Figure 1. It was explained

15 that the amounts of expressions of NGalNac-T1 and NGalNac-T2 were high in the nervous system, stomach and spermary, respectively.

#### Example 4 Expression analysis of human cancerous tissue

20

The expression levels of both genes of human lung cancerous tissue and normal lung tissue in the same patient were analyzed. The methods were the same as that of Example 3, provided that b-actin gene was used as a control gene,  
25 and Pre-Developed TaqMan Assay Reagents Endogenous Control Human Beta-actin (Applied Biosystems Co.) was used in the quantification (Figure 2). Consequently, it was explained that both genes can be used at least as a lung cancer marker.

Example 5 Assay for acceptor substrates of glycosyl-transferase activities

For the reaction of GalNAc-T assay, 50 mM MES buffer  
5 (pH 6.5) containing 0.1 % triton X-100, 1 mM UDP-GalNAc, 10  
mM MnCl<sub>2</sub> and 500 µM each acceptor substrate was used. A 10  
µl of enzyme solution for 20 µl of each reaction mixture  
were added and incubated at 37 °C for various periods.  
After the incubation the mixture was filtrated with  
10 Ultrafree-MC column (Millipore, Bedford, MA), and 10 µl  
aliquot was subjected to reversed-phase high performance  
liquid chromatography (HPLC) on an ODS-80Ts QA column (4.6 x  
250 mm; Tosoh, Tokyo, Japan). A 0.1 % TFA/H<sub>2</sub>O with 12 %  
acetonitrile was used as a running solution. An ultraviolet  
15 spectrophotometer (absorbance at 210 nm), SPD-10A<sub>VP</sub> (Shimazu,  
Kyoto, Japan) was used for detection of the peaks. When the  
pyridyl amino-labeled oligosaccharides were utilized as  
acceptor substrates, 50 nM substrates were added into the  
reaction mixtures. For the analyses of the products derived  
20 from pyridyl amino labeled oligosaccharides, 100 mM acetic  
acid/triethylamine (pH4.0) was used as a running solution  
and the products were eluted with a 30-70% gradient of 1% 1-  
butanol in running solution at a flow rate of 1.0 ml/min at  
55 °C.  
25 A 200 µg of the reaction product was dissolved in 150  
µl of D<sub>2</sub>O using a micro cell and used as a sample for <sup>1</sup>H NMR  
experiments. One-dimensional and two-dimensional <sup>1</sup>H NMR  
spectra were recorded with DMX750 (Bruker, Germany, 750.13  
MHz for <sup>1</sup>H nucleus) and ECA800 (JEOL, Tokyo, Japan, 800.14

MHz for  $^1\text{H}$  nucleus) spectrometers at 25 °C. Methylene proton of benzyl group in higher field (4.576 ppm) was used as a reference for the  $^1\text{H}$  NMR chemical shifts tentatively.

To investigate the specificity for acceptor substrates, N- and O-glycans containing GlcNAc on their non-reducing termini were utilized. As shown in Table 6 and 7, all acceptor substrates examined could receive a GalNAc residue.

10 Table 6

Substrate specificity of NGalNAc-Ts			
Acceptor substrate	Relative activity (%)		
	NGalNAc-T1	NGalNAc-T2	
1. GlcNAc $\beta$ -Bz	100	100	
2. GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$ -pNp (core2-pNp)	15.2	11.4	
3. GlcNAc $\beta$ 1-3GalNAc $\alpha$ -pNp (core3-pNp)	20.0	32.3	
4. GlcNAc $\beta$ 1-6GalNAc $\alpha$ -pNp (core6-pNp)	190.7	220.4	

15

20

Table 7

Substrate specificity of NGalNAc-Ts			
Acceptor substrate		Relative activity (%)	
		NGalNAc-T1	NGalNAc-T2
1.	$\begin{array}{c} \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-6 \\ \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-3 \end{array} \text{Man}\beta 1-4\text{GlcNAc}\beta 1-4 \text{ GlcNAc-PA}$	100	100
2.	$\begin{array}{c} \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-6 \\ \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-3 \end{array} \text{Man}\beta 1-4\text{GlcNAc}\beta 1-4 \text{ GlcNAc-PA}$	76.8	87.1
3.	$\begin{array}{c} \text{Gal}\beta 1-4\text{GlcNAc}\beta 1-2\text{Man}\alpha 1-6 \\ \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-3 \end{array} \text{Man}\beta 1-4\text{GlcNAc}\beta 1-4 \text{ GlcNAc-PA}$	26.2	45.0
4.	$\begin{array}{c} \text{Gal}\beta 1-4\text{GlcNAc}\beta 1-2\text{Man}\alpha 1-6 \\ \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-3 \end{array} \text{Man}\beta 1-4\text{GlcNAc}\beta 1-4 \text{ GlcNAc-PA}$	26.7	51.7
5.	$\begin{array}{c} \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-6 \\ \text{Gal}\beta 1-4\text{GlcNAc}\beta 1-2\text{Man}\alpha 1-3 \end{array} \text{Man}\beta 1-4\text{GlcNAc}\beta 1-4 \text{ GlcNAc-PA}$	16.2	21.6
6.	$\begin{array}{c} \text{GlcNAc}\beta 1-2\text{Man}\alpha 1-6 \\ \text{Gal}\beta 1-4\text{GlcNAc}\beta 1-2\text{Man}\alpha 1-3 \end{array} \text{Man}\beta 1-4\text{GlcNAc}\beta 1-4 \text{ GlcNAc-PA}$	3.4	5.0

<sup>1</sup>H NMR spectroscopy was performed to determine the newly formed glycosidic linkage of NGalNAc-T2 product.

- 5 One-dimensional <sup>1</sup>H NMR spectrum of the NGalNAc-T2 product is shown in Fig. 5. In the NMR spectra, signal integrals (not shown, five phenyl protons of Bz, two methylene protons of Bz, two anomeric protons, twelve sugar protons except anomeric protons, six methyl protons of two N-acetyl groups)
- 10 were in good correspondence with the structure of GalNAc-GlcNAc-O-Bz. As shown in Fig. 5 and in Table 8, two anomeric protons revealed resonances at very close magnetic field with coupling constant ( $J_{1,2}$ ) larger than 8 Hz. This indicates that two pyranoses in the samples are in
- 15  $\beta$ -gluco-configuration. All <sup>1</sup>H signals could be assigned after high resolutional detections of COSY, TOCSY and NOESY

experiments. The anomeric resonance in the lower field showed NOE with two methylene protons of benzyl group in the sample (not shown), on the other hand, the anomeric resonance in higher field did not show NOE with methylene protons (not shown). The facts mean that the anomeric resonance in the lower field is responsible for the anomeric proton of the substrate pyranose ( $\beta$ -GlcNAc, defined as A), and that the anomeric proton in the higher field corresponds to anomeric proton of the transferred pyranose ( $\beta$ -GalNAc, defined as B). The chemical shifts and coupling constants of sugar part of the sample were shown in Table 8. The chemical shift and signal splitting of B-4 resonance was characteristic in  $\beta$ -Gal configuration [see Reference 15], and the order in chemical shift of A1-A6 protons was characteristically similar to observed spectrum of  $\beta$ -GlcNAc in LNnT (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc). As shown in Fig. 6, weak NOE cross peak between B1 and A4 and very weak NOE cross peaks between B1 and two A6 were observed in addition to strong inner residual NOEs between B1 and B5 and between A1 and A5. These suggest the existence of  $\beta$ 1-4 linkage between two pyranoses. Results in NMR experiments thus indicated clearly that the product by NGalNAc-T2 is GalNAc $\beta$ 1-4GlcNAc-O-Bz.

Table 8

*Chemical shifts (ppm) and coupling constants (Hz) of sugar CH protons in the NGalNAc-T2 product*

	NGalNAc-T2 product	
	GlcNAc	GalNAc
<sup>1</sup> H Chemical shifts (ppm) <sup>a</sup>		
δ1	4.434	4.425
δ2	3.647	3.831
δ3	3.546	3.665
δ4	3.534	3.846
δ5	3.411	3.628
δ6	3.589	3.696
δ6	3.782	3.680
δCH <sub>3</sub>	1.830	1.987
Coupling constants (Hz)		
J <sub>1,2</sub>	8.5	8.4
J <sub>2,3</sub>		10.8
J <sub>4,5</sub>		<3.7
J <sub>5,6a</sub>	5.6	<3.7
J <sub>5,6b</sub>	2.0	
J <sub>6a,6b</sub>	12.1	

<sup>a</sup>, The chemical shifts were set as the higher field signal of the benzyl methylene protons is ppm tentatively.

5 Example 6 LacdiNAc synthesizing activity of NGalNAc-T2  
toward asialo/agalacto-fetal calf fetuin

As demonstrated in Table 6 and 7, both NGalNAc-T1 and  
-T2 transferred GalNAc toward both O- and N-glycans  
10 substrates. The LacdiNAc (GalNAcβ1-4GlcNAc) structures have  
been found in N-glycans of some glycoproteins in human.  
Therefore, to determine the activity of NGalNAc-T2 to  
transfer GalNAc to a glycoprotein, fetal calf fetuin (FCF),  
which has both N- and O-glycans, was utilized as an acceptor  
15 substrate.

Fetal calf fetuin (FCF), neuraminidase,  $\beta$ 1-4 galactosidase and glycopeptidase F were purchased from Sigma, Nacalai Tesque (Kyoto, Japan), Calbiochem and Takara, respectively. Asialo/agalacto-FCF was prepared from 200  $\mu$ g of FCF by incubating with 4  $\mu$ U of neuraminidase and 12  $\mu$ U of  $\beta$ 1,4-galactosidase at 37 °C for 16 hr. The transfer of GalNAc by GalNAc-T2 to glycoprotein was performed in 20  $\mu$ l of a standard reaction mixture containing 50  $\mu$ g of asialo/agalacto-FCF produced by glycosidase treatment.

After the incubation at 37°C for 16 hr, each 5  $\mu$ l of the reaction mixture was digested with glycopeptidase F (GPF) according to manufacture's instruction. For detection of transferred GalNAc, horseradish peroxidase (HRP) conjugated lectin, Wisteria floribunda agglutinin (WFA) (EY Laboratories, San Mateo, CA), was used. A 1  $\mu$ l of reaction mixtures subjected to 12.5% SDS-PAGE were transferred to nitrocellulose membrane (Schleicher & Schuell, Keene, NH) and stained with 0.1% HRP conjugated WFA lectin. The signals were detected using enhanced chemiluminescence (ECL) and Hyperfilm ECL (Amersham Biosciences).

As shown in Fig. 3, asialo/agalacto-FCF appeared as approximately 55 and 60 kDa band (lane 1). NGalNAc-T2 effectively transferred GalNAc to asialo/agalacto-FCF (lane 5). Furthermore, the band mostly disappeared by a GPF treatment, and its molecular size was detected at approximately 45 and 50 kDa position by Coomassie staining (Fig. 3, lane 3 and 6). In the case of NGalNAc-T1, the activity toward asialo/agalacto-FCF was same as NGalNAc-T2 (data not shown).



Example 7 Analysis of N-glycan structures on glycodeilin  
from NGalNAc-T1 and -T2 gene transfected CHO cells

5           As shown above, both NGalNAc-T1 and -T2 could  
synthesize LacdiNAc structures on mono- and oligosaccharide  
acceptors. Actually, it is known that the LacdiNAc  
structures exist in N-glycans on some glycoproteins.  
Therefore we examined the ability of NGalNAc-T1 to construct  
10 LacdiNAc on glycodeilin, which is one of major glycoproteins  
carrying LacdiNAc structures, *in vivo*. CHO cells were  
employed for this purpose, because glycodeilin produced in  
CHO cells is devoid of any of the LacdiNAc-based chains.

          The glycodeilin expression vector was transfected into  
15 CHO cells expressing NGalNAc-T1 or -T2 gene and the culture  
medium was collected from 48 hr-culture medium. Glycodeilin  
was harvested with WFA affinity column from the culture  
medium. The harvested glycodeilin was applied to SDS-PAGE  
and used for lectin blotting with WFA.

20           As shown in Fig. 7, the non-reducing terminal GalNAc  
was detected only when NGalNAc-T1 or -T2 gene was co-  
transfected with glycodeilin gene. These bands were  
disappeared by N-glycanase<sup>TM</sup> treatment, therefore these  
GalNAc residues might exist in N-glycans.

25           Example 8 Preparation of mouse proteins of the present  
invention

1. Search through a genetic database and determination of

the nucleic acid sequence of a novel mouse  
N-acetylgalactosaminyltransferase

A search of similar genes through a mouse genomic  
database (UCSC Human Genome Project, Nov. 2001 mouse  
5 assembly archived Sep. 15, 2002,  
<http://genome-archive.cse.ucsc.edu/>) was performed by use of  
the genes for existing human NGalNAc-T1 and -T2. The  
sequences used were SEQ ID NOs: 1, 3, 26 and 28. The search  
was performed using a program such as Blast [Altschul et  
10 al., J. Mol. Biol., 215, 403-410 (1990)].

As a result, two homologous genes were found on  
mouse chromosome 7 and 6. The nucleotide and amino acid  
sequences of the first gene on chromosome 7, which is an  
ortholog of human NGalNAc-T1, were shown as SEQ ID NOs: 26  
15 and 28. The second ones on chromosome 6 were described as  
SEQ ID NOs: 27 and 29.

## 2. Integration of GalNAc-T genes into an expression vector

To prepare each expression system of mouse  
20 NGalNAc-T, a portion of each gene was first integrated into  
pFLAG-CMV1 (Sigma).

### Integration of mNGalNAc-T1 into pFLAG-CMV1

The mouse NGalNAc-T2 (mNGalNAc-T2) gene encoding its  
25 putative catalytic domain (amino acid 45 to 1,034) was  
amplified with two primers, 5'-CCC AAG CTT CGC CTG GGC TAC  
GGG CGA GAT-3' (SEQ ID NO: 31) and 5'-GCT CTA GAC TCA GGA  
TCG CTG TGC GCG GGC A-3' (SEQ ID NO: 32), using the cDNA  
derived from mouse brain as a template. The mRNA was

prepared from mouse brain with RNeasy mini kit (Qiagen), then the cDNA was synthesized with SuperScript first-strand synthesis system for RT-PCR (Invitrogen). For the PCR, LA Taq DNA polymerase (Takara) was used. The amplified 2.7 kb  
5 fragment was digested with endonuclease Hind III and Xba I, then the digested fragment was inserted into pFLAG-CMV-1 and pFLAG-mNGalNAc-T1 was constructed.

#### Integration of mNGalNAc-T2 into pFLAG-CMAV1

10 The mouse NGalNAc-T2 (mNGalNAc-T2) gene encoding its putative catalytic domain (amino acid 57 to 986) was amplified with two primers, 5'-CCC AAG CTT CGG CCC AGG CCG GCG GGA ACC-3' (SEQ ID NO: 33) and 5'-GGA ATT CTC ACG GCA TCT TCA TTT GGC GA-3' (SEQ ID NO: 34), using the cDNA  
15 derived from mouse stomach as a template. The mRNA was prepared from mouse stomach with RNeasy mini kit (Qiagen), then the cDNA was synthesized with SuperScript first-strand synthesis system for RT-PCR (Invitrogen). For the PCR, LA Taq DNA polymerase (Takara) was used. The amplified 2.7 kb  
20 fragment was digested with endonuclease Hind III and EcoR I, then the digested fragment was inserted into pFLAG-CMV-1 and pFLAG-mNGalNAc-T2 was constructed.

#### 3. Transfection and expression of recombinant enzymes

25 A 15 µg of pFLAG-mNGalNAc-T1 or pFLAG-mNGalNAc-T2 was induced into  $2 \times 10^6$  of HEK293T cells which were cultured overnight in DMEM (Dulbecco's modified Eagle's medium) including 10 % FCS (fetal calf serum), using Lipofectamine 2000 (Invitrogen Co.) as a protocol provided

by the same company. A supernatant of 48-72 hours was collected. The supernatant was mixed with  $\text{NaN}_3$  (0.05 %),  $\text{NaCl}$  (150 mM,  $\text{CaCl}_2$  (2 mM) and an anti-M1 resin (Sigma Co.) (50  $\mu\text{l}$ ), and the mixture was stirred overnight (3000 rpm, 5 min, 4 °C) to collect a pellet. The pellet was combined with 900  $\mu\text{l}$  of 2 mM  $\text{CaCl}_2$ /TBS and re-centrifuged (2000 rpm, 5 min, 4 °C), after which the pellet was suspended in 200  $\mu\text{l}$  of 1 mM  $\text{CaCl}_2$ /TBS to give a sample for assaying activity (mNGalNAc-T1 or mNGalNAc-T2 enzyme solution).

The enzyme was subjected to conventional SDS-PAGE and Western blotting, and the expression of the intended protein was confirmed. Anti-FLAG M2-peroxydase (A-8592, SIGAIA Co.) was used as an antibody.

## References

1. Sugita, M., S. Itonori, F. Inagaki and T. Hori, Characterization of two glucuronic acid-containing glycosphingolipids in larvae of the green-bottle fly, *Lucilia caesar*. J. Biol. Chem., 1989. 264, p.15028-33
2. Helling, F., R.D. Dennis, B. Weske, G. Nores, J. Peter-Katalinic, U. Dabrowsli, H. Egge and H. Wiegandt, Glycosphingolipids in insects. The amphoteric moiety, N-acetylglucosamine-linked phosphoethanolamine, distinguishes a group of ceramide oligosaccharides from the pupae of *Calliphora vicina* (Insecta: Diptera). Eur. J. Biochem., 1991. 200, p.409-21
3. Weisshaar, G., J. Hiyama, A.G. Renwick and M. Nimtz, NMR investigations of the N-linked oligosaccharides at individual glycosylation sites of human lutropin. Eur. J.

Biochem., 1991. 195, p.257-68

4. White, T., E.P. Bennet, K. Takio, T. Sorenesen, N.

Bonding and H. Clausen, Purification and cDNA cloning of a human UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-

5 acetylgalactosaminyltransferase. J. Biol. Chem., 1995. 270, p.24156-65

5. Bennett, E.P., H. Hassan and H. Clausen, cDNA cloning and expression of a novel human UDP-N-acetyl-alpha-D-galactosamine. Polypeptide N-

10 acetylgalactosaminyltransferase, GalNAc-t3. J. Biol. Chem., 1996. 271, p.17006-12

6. Bennett, E.P., H. Hassan, U. Mandel, E. Mirgorodskaya, P. Roepstorff, J. Burchell, J. Taylor-Papadimitriou, M.A.

Hollingsworth, G. Merckx, A.G. van Kessel, H. Eiberg, R.

15 Steffensen and H. Clausen, Cloning of a human UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-

acetylgalactosaminyltransferase that complements other GalNAc-transferases in complete O-glycosylation of the MUC1 tandem repeat. J. Biol. Chem., 1998. 273, p.30472-81

20 7. Bennett, E.P., H. Hassan, J. Mandel, M.A. Hollingsworth, N. Akisawa, Y. Ikematsu, G. Merckx, A.G. van Kessel, S.

Olofsson and H. Clausen, Cloning and characterization of a close homologue of human UDP-N-acetyl-alpha-D-

galactosamine:polypeptide N-acetylgalactosaminyltransferase-

25 T3, designed GalNAc-T6. Evidence for genetic but not functional redundancy. J. Biol. Chem., 1999. 274, p.25362-70

8. Bennett, E.P., H. Hassan, M.A. Hollingsworth and H.

Clausen, A novel human UDP-N-acetyl-D-

galactosamine:polypeptide N-acetylgalactosaminyltransferase,

GalNAc-T7, with specificity for partial GalNAc-glycosylated acceptor substrates. FEBS Lett., 1999. 460, p.226-30

9. White, K. E., B. Lorenz, T. Meitinger, T.M. Strom and M.J. Econs, Gene, 2000. 246, p.347-56

5 10. Toba, S., M. Tenno, M. Konishi, T. Mikami, N. Itoh and A. Kurosaka, Brain-specific expression of a novel human UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase (GalNAc-T9). Biochim. Biophys. Acta., 2000. 7, p.264-8

11. Nagata, Y., S. Yamashiro, J. Yodoi, K.O. Lloyd, H. Shiku  
10 and K. Furukawa, Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides. J. Biol. Chem., 1992. 269, p.12082-9

12. Yamamoto, F., J. Marken, T. Tsuji, T. White, H. Clausen  
15 and S. Hakomori, Cloning and characterization of DNA complementary to human UDP-GalNAc:Fuc alpha 1----2Gal alpha 1----3GalNAc transferase (histo-blood group A transferase) mRNA. J. Biol. Chem., 1990. 265, p.1146-51

13. Xu, H., T. Storch, M. Yu, S.P. Elliott and D.B. Haslam,  
20 Characterization of the human Forssman synthetase gene. An evolving association between glycolipid synthesis and host-microbial interactions. J. Biol. Chem., 1999. 274, p.29390-8

14. Guo, J.M., Y. Zhang, L. Cheng, H. Iwasaki, H. Wang, T. Kubota, K. Tachibana and H. Narimatsu, Molecular cloning and  
25 characterization of a novel member of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase family, pp-GalNAc-T12(1). FEBS Lett., 2002. 524, p.211-8

15. Ishizuka, Y., T. Nemoto, M. Fujiwara, K. Fujita and H. Nakanish, Three-dimensional structure of fucosyllactoses in

an aqueous solution. J. Carbohydr. Chem., 1999. 18, p.523-33

#### Industrial Applicability

According to the present invention, an enzyme which  
5 transfers N-acetylgalactosamine to N-acetylglucosamine via a  
 $\beta$ 1-4 linkage was isolated and the structure of its gene was  
explained. This led to the production of said enzyme or the  
like by genetic engineering techniques, the production of  
oligosaccharides using said enzyme, and the diagnosis of  
10 diseases on the basis of said gene or the like.

## CLAIMS

1. A isolated protein having an amino acid sequence which is selected from a group consisting of SEQ ID NOs: 1, 3, 26 and 28 or a variant of said amino acid sequence, wherein one or more amino acids are substituted or deleted, or one or more amino acids are inserted or added, having the activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a  $\beta$ 1-4 linkage.

2. The protein of Claim 1, wherein the amino acid sequence is shown in SEQ ID NO: 1 or 3.

3. The protein of Claim 1, wherein the amino acid sequence is shown in SEQ ID NO: 26 or 28.

4. The protein of Claim 1 having an identity of 50 % or more to the amino acid sequence shown in SEQ ID NO: 1 or 26.

5. The protein of Claim 1 having an identity of 60 % or more to the amino acid sequence shown in SEQ ID NO: 1 or 26.

6. A isolated nucleic acid encoding the protein of any one of Claims 1 to 5.

7. A nucleic acid encoding the protein of Claim 1 or 2, which hybridizes with a nucleic acid having the nucleotide sequence shown in SEQ ID NO: 2 or 4 under stringent conditions.

8. A nucleic acid encoding the protein of Claim 1 or 3, which hybridizes with a nucleic acid having the nucleotide sequence shown in SEQ ID NO: 27 or 29 under stringent conditions.

9. The nucleic acid of Claim 7 having a nucleotide sequence represented by nucleotides 1-3120 of the nucleic



acid sequence shown in SEQ ID NO: 2 or nucleotides 1-2997 of the nucleic acid sequence shown in SEQ ID NO: 4.

10. The nucleic acid of Claim 8 having a nucleotide sequence represented by nucleotides 1-3105 of the nucleic acid sequence shown in SEQ ID NO: 27 or nucleotides 1-2961 of the nucleic acid sequence shown in SEQ ID NO: 29.

11. A recombinant vector containing the nucleic acid of any one of Claims 6 to 10 and being capable of expressing said nucleic acid in a host cell.

12. A host cell transformed with the recombinant vector of Claim 11.

13. An analytical nucleic acid, which hybridizes to the nucleic acid of Claim 6 under stringent conditions.

14. The analytical nucleic acid of Claim 13, which is used as a primer and is selected from a group consisting of SEQ ID NOs: 20, 21, 23 and 24.

15. The analytical nucleic acid of Claim 13, which is used as a probe and is SEQ ID NO: 22 or 25.

16. The analytical nucleic acid of Claim 13, which is used as a cancer marker.

17. An assay kit comprising the analytical nucleic acid of any one of Claims 14 to 16 and assay instructions.

18. An antibody binding to the protein of any one of Claims 1 to 5.

19. The antibody of Claim 18, which is an monoclonal antibody.

20. A method for determining a canceration of a biological sample comprising the steps of:

(a) quantifying the protein of any one of Claims 1 to 5 in

the biological sample; and

(b) estimating that the biological sample is cancerous in a case that the quantity value of said protein in the biological sample is more than that in a control biological sample.

21. The method of Claim 20, wherein said protein is quantified by use of the antibody of Claims 18 or 19.

22. A method for determining a canceration of a biological sample comprising the steps of:

(a) quantifying the nucleic acid of Claim 6 in the biological sample; and

(b) estimating that the biological sample is cancerous in a case that the quantity value of the nucleic acid of Claim 6 in the biological sample is 1.5 times or more than that in a control biological sample.

23. The method of Claim 22, comprising the steps of:

(a) hybridizing at least one of the analytical nucleic acids of Claim 13 to the nucleic acid of Claim 6 in the biological sample;

(b) amplifying the nucleic acid of Claim 6;

(c) hybridizing the analytical nucleic acids of Claim 13 to the amplification product;

(d) quantifying a signal rising from said amplification product and said analytical nucleic acid hybridized; and

(e) estimating that the biological sample is cancerous in the case that the quantity value of said signal is 1.5 times or more than that of a corresponding signal of a control biological sample.

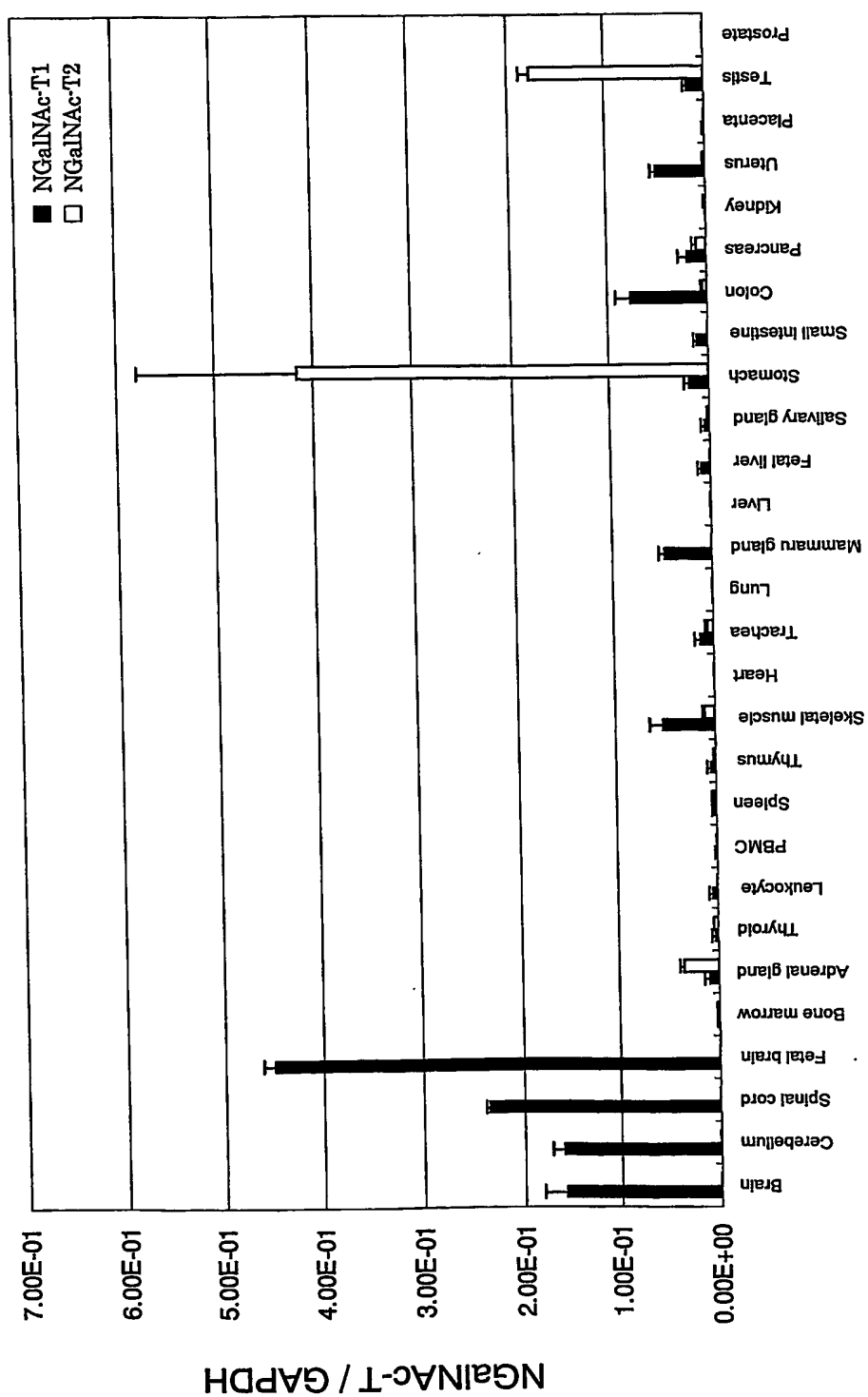
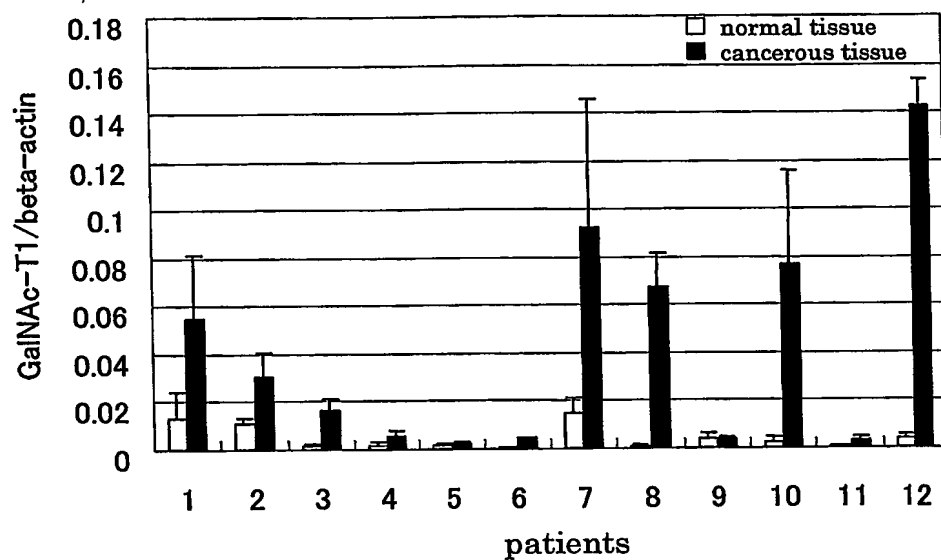


Fig. 1

A

GalNAc-T1



B

GalNAc-T2

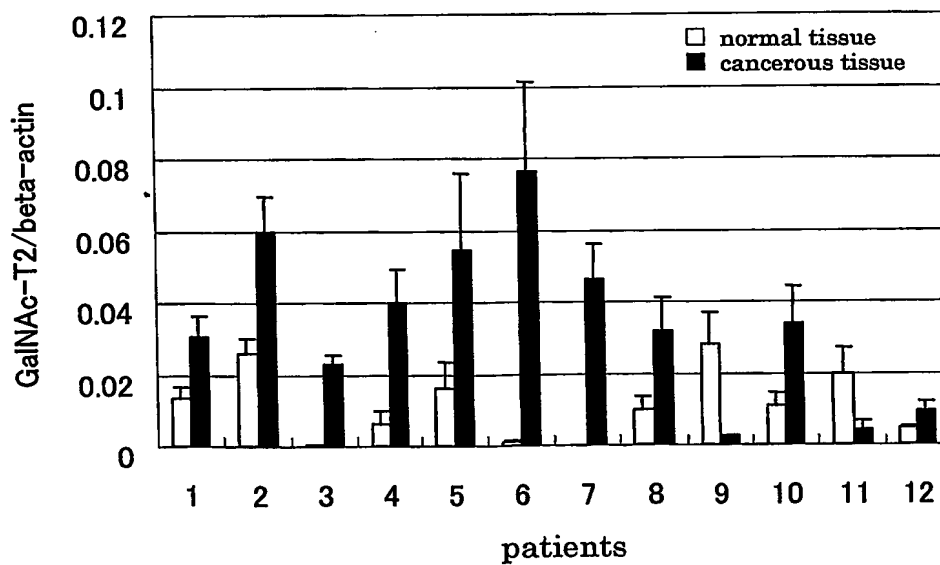
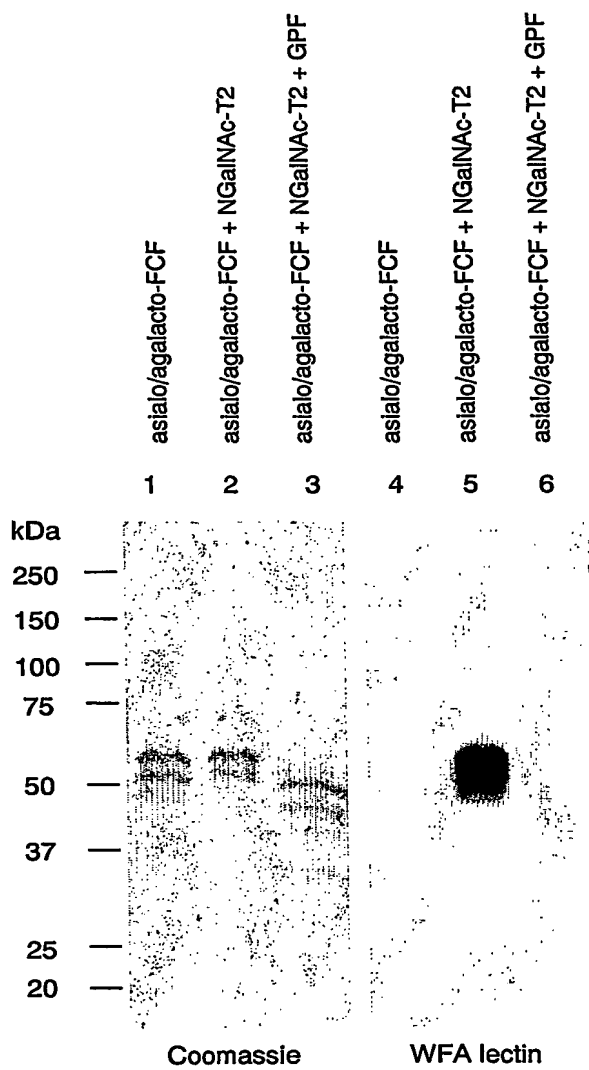


Fig. 2

**Fig. 3**

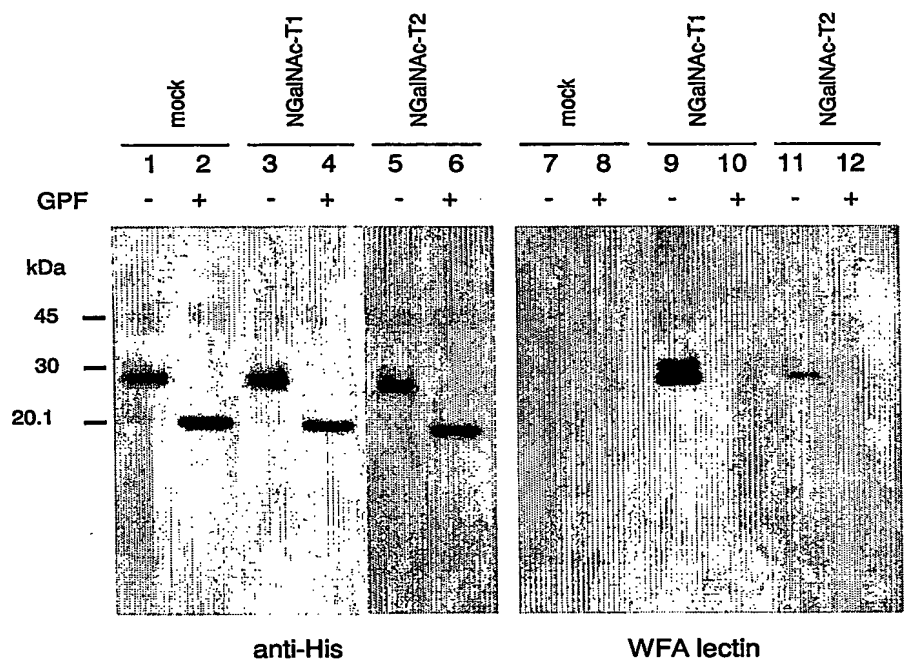
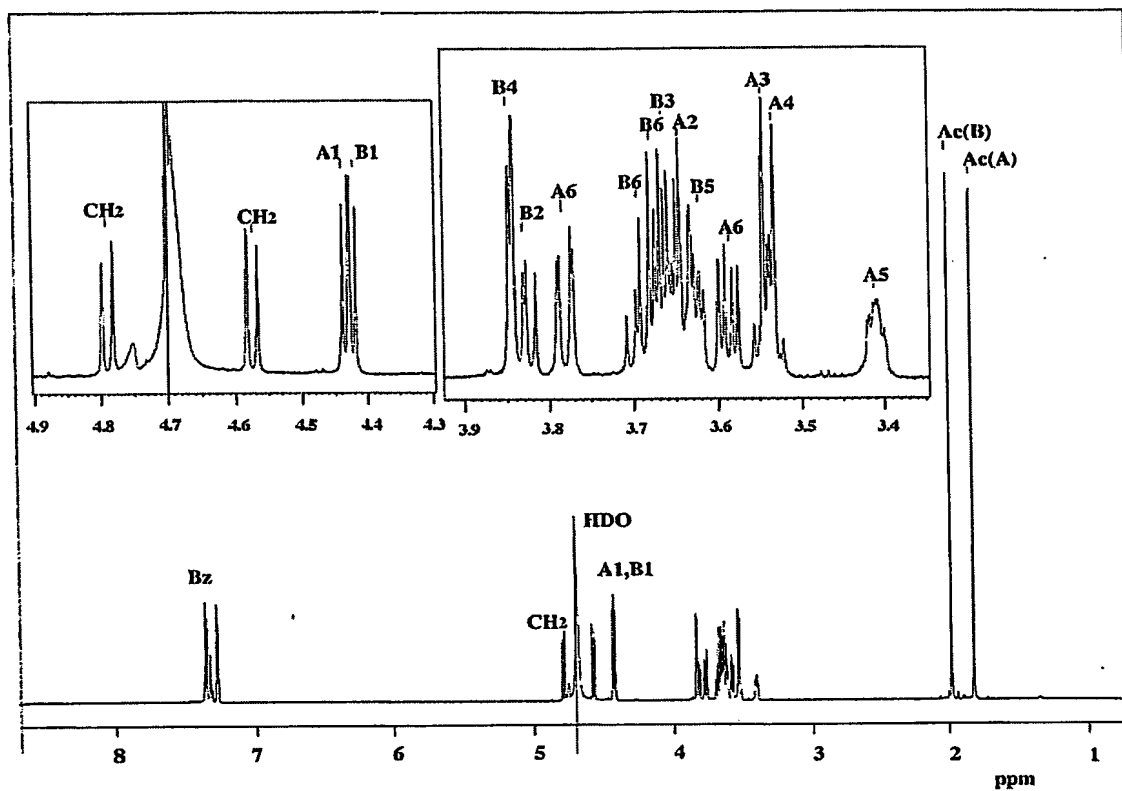
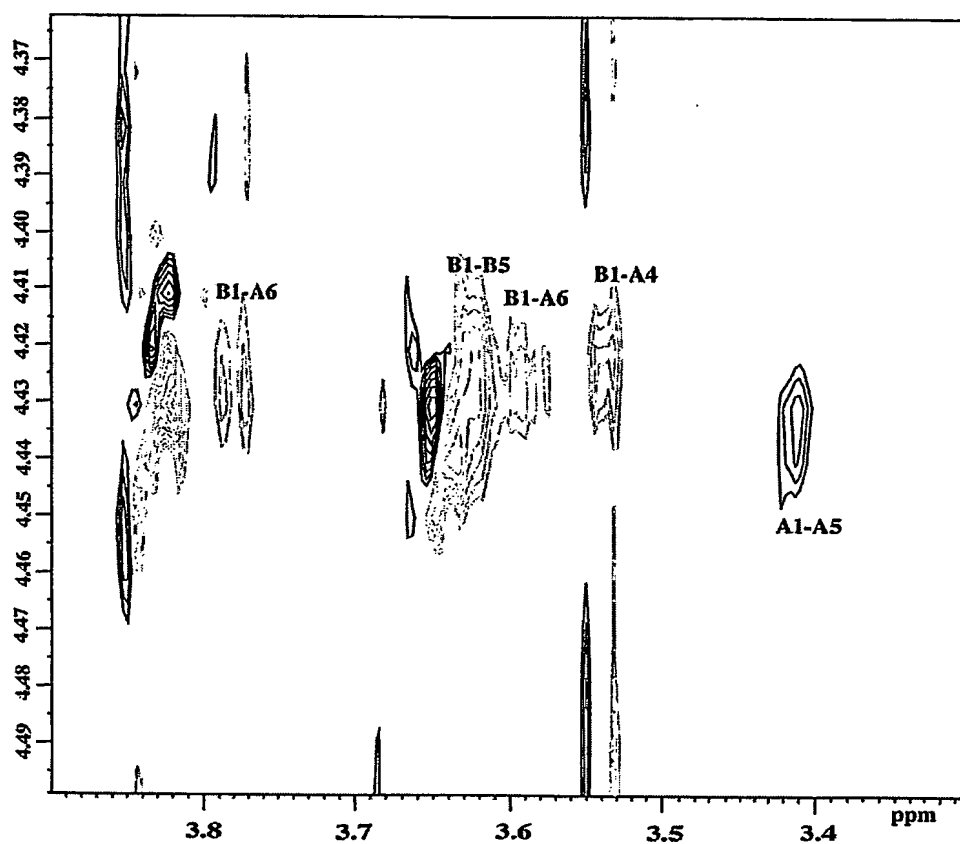


Fig. 4

**Fig. 5**

**Fig. 6**



## SEQUENCE LISTING

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AMERSHAM BIOSCIENCES KK

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720

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725

730

735

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740

745

750

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Asp Pro Arg Asp Thr Phe Phe Leu Thr Pro Arg Met Glu Ser Ser Ser  
325 330 335

ctg gag aac gtg ctg gag ccc tgc gcc tac gcc ccc acc tac gtg gtc 1056  
Leu Glu Asn Val Leu Glu Pro Cys Ala Tyr Ala Pro Thr Tyr Val Val  
340 345 350

aag gac ttc ccg atc gcc aga tac cag ggc ctg caa ttt gtg tac ctg 1104  
Lys Asp Phe Pro Ile Ala Arg Tyr Gln Gly Leu Gln Phe Val Tyr Leu  
355 360 365

tcc ttc gtt tat ccc aac gac tac act cgc ctc acc cac atg gag acg 1152  
Ser Phe Val Tyr Pro Asn Asp Tyr Thr Arg Leu Thr His Met Glu Thr  
370 375 380

gac aac aag tgc ttc tac cgc gag tct ccg ctg tat ctg gag agg ttt 1200

Asp Asn Lys Cys Phe Tyr Arg Glu Ser Pro Leu Tyr Leu Glu Arg Phe  
385 390 395 400

ggg ttc tat aaa tac atg aag atg gac aag gag gag ggg gat gag gat 1248  
Gly Phe Tyr Lys Tyr Met Lys Met Asp Lys Glu Glu Gly Asp Glu Asp  
405 410 415

gaa gaa gac gag gtg cag cgc cga gcc ttc ctc ttc ctc aac ccg gac 1296  
Glu Glu Asp Glu Val Gln Arg Arg Ala Phe Leu Phe Leu Asn Pro Asp  
420 425 430

gac ttc ctg gac gac gag gac gag ggg gag ctg ctc gac agc ctg gag 1344  
Asp Phe Leu Asp Asp Glu Asp Glu Gly Glu Leu Leu Asp Ser Leu Glu  
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ccc acc gag gcg gcc ccg ccc agg agc ggc ccc cag tcc ccc gcc cca 1392  
Pro Thr Glu Ala Ala Pro Pro Arg Ser Gly Pro Gln Ser Pro Ala Pro  
450 455 460

gca gcc ccc gcc cag ccc gga gcc acc ctc gcc ccg ccg acc cct ccc 1440  
Ala Ala Pro Ala Gln Pro Gly Ala Thr Leu Ala Pro Pro Thr Pro Pro  
465 470 475 480

cgc ccc cgg gac ggg ggg acc ccc agg cac tcc cgg gcc ctg agc tgg 1488  
Arg Pro Arg Asp Gly Gly Thr Pro Arg His Ser Arg Ala Leu Ser Trp  
485 490 495

gcc gcc agg gcc gcc cgc cct ttg ccg ctc ttc ttg ggc cga gct ccg 1536  
Ala Ala Arg Ala Ala Arg Pro Leu Pro Leu Phe Leu Gly Arg Ala Pro

500	505	510	
ccc ccg cgc cct gca gtg gag cag ccg ccc cca aag gtg tac gtg acc			1584
Pro Pro Arg Pro Ala Val Glu Gln Pro Pro Pro Lys Val Tyr Val Thr			
515	520	525	
agg gtg cgg ccg gga cag cgg gca tcc ccc cgg gcc cca gcg ccg cgt			1632
Arg Val Arg Pro Gly Gln Arg Ala Ser Pro Arg Ala Pro Ala Pro Arg			
530	535	540	
gcg ccc tgg ccg ccc ttc cct ggc gtc ttc ctg cac ccc agg cct ctg			1680
Ala Pro Trp Pro Pro Phe Pro Gly Val Phe Leu His Pro Arg Pro Leu			
545	550	555	560
ccc aga gtg cag ctg cgg gcg ccc cca cgc cca ccc cgg ccc cac ggc			1728
Pro Arg Val Gln Leu Arg Ala Pro Pro Arg Pro Pro Arg Pro His Gly			
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cgc agg acc ggc ggc ccc cag gcc aca cag ccg agg ccc cca gcc cgg			1776
Arg Arg Thr Gly Gly Pro Gln Ala Thr Gln Pro Arg Pro Pro Ala Arg			
580	585	590	
gcg cag gcc acc caa ggg ggc cgg gag ggc cag gcg cgc acg ctg gga			1824
Ala Gln Ala Thr Gln Gly Gly Arg Glu Gly Gln Ala Arg Thr Leu Gly			
595	600	605	
cct gcg gcg ccc aca gtg gac tca aac ttg tcc tcc gaa gcg cgg ccc			1872
Pro Ala Ala Pro Thr Val Asp Ser Asn Leu Ser Ser Glu Ala Arg Pro			
610	615	620	

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Val Thr Ser Phe Leu Ser Leu Ser Gln Val Ser Gly Pro Gln Leu Pro  
625 630 635 640

ggg gag ggc gaa gag gag gag gaa ggg gag gac gat ggg gcc ccg ggc 1968  
Gly Glu Gly Glu Glu Glu Glu Gly Glu Asp Asp Gly Ala Pro Gly  
645 650 655

gac gag gcc gcg tcg gag gac agc gag gag gcc gcg ggc ccg gcg ctc 2016  
Asp Glu Ala Ala Ser Glu Asp Ser Glu Glu Ala Ala Gly Pro Ala Leu  
660 665 670

gga cgc tgg cgt gag gac gcc atc gac tgg cag cgc acg ttc agc gtg 2064  
Gly Arg Trp Arg Glu Asp Ala Ile Asp Trp Gln Arg Thr Phe Ser Val  
675 680 685

ggc gcc gtg gac ttc gag ctg ctg cgc tcg gac tgg aac gac ctg cga 2112  
Gly Ala Val Asp Phe Glu Leu Leu Arg Ser Asp Trp Asn Asp Leu Arg  
690 695 700

tgc aac gtt tcg ggg aac ctg cag ctg ccg gag gcg gag gcc gtg gac 2160  
Cys Asn Val Ser Gly Asn Leu Gln Leu Pro Glu Ala Glu Ala Val Asp  
705 710 715 720

gtg acc gct cag tac atg gag cgg ctg aac gcg cgc cac ggc ggg cgc 2208  
Val Thr Ala Gln Tyr Met Glu Arg Leu Asn Ala Arg His Gly Gly Arg  
725 730 735

ttc gcg ctt ctg cgc atc gtg aac gtg gag aag cgc cgg gac tcg gcg 2256  
Phe Ala Leu Leu Arg Ile Val Asn Val Glu Lys Arg Arg Asp Ser Ala  
740 745 750

cga ggg agt cgc ttc ctg ctg gag ctg gag ctg cag gag cgc ggg ggc 2304  
Arg Gly Ser Arg Phe Leu Leu Glu Leu Glu Leu Gln Glu Arg Gly Gly  
755 760 765

ggc cgc ctg cga ctg tcc gag tac gtc ttc ctg cgg ctg ccg gga gcc 2352  
Gly Arg Leu Arg Leu Ser Glu Tyr Val Phe Leu Arg Leu Pro Gly Ala  
770 775 780

cgc gta ggg gat gca gac gga gaa agt ccc gaa ccc gct ccc gcc gcc 2400  
Arg Val Gly Asp Ala Asp Gly Glu Ser Pro Glu Pro Ala Pro Ala Ala  
785 790 795 800

tcc gtg cgc ccc gac ggc cgc ccc gag ctc tgc cgg cca ctg cgc ctg 2448  
Ser Val Arg Pro Asp Gly Arg Pro Glu Leu Cys Arg Pro Leu Arg Leu  
805 810 815

gcc tgg cgc cag gac gtg atg gtt cac ttc atc gtg cca gtg aaa aac 2496  
Ala Trp Arg Gln Asp Val Met Val His Phe Ile Val Pro Val Lys Asn  
820 825 830

cag gca cgg tgg gtg gca cag ttc ctg gcg gac atg gct gcg ctg cac 2544  
Gln Ala Arg Trp Val Ala Gln Phe Leu Ala Asp Met Ala Ala Leu His  
835 840 845

gcg cgc acc ggg gac tcg cgt ttc agc gtc gtc ctg gtg gat ttc gag 2592

Ala Arg Thr Gly Asp Ser Arg Phe Ser Val Val Leu Val Asp Phe Glu  
850 855 860

agc gag gat atg gac gtg gag cgg gcc ctg cgc gcc gcg cgc ctg ccc 2640  
Ser Glu Asp Met Asp Val Glu Arg Ala Leu Arg Ala Ala Arg Leu Pro  
865 870 875 880

cgg tac cag tac ctg aga cga acc ggg aac ttc gag cgc tcc gcc ggg 2688  
Arg Tyr Gln Tyr Leu Arg Arg Thr Gly Asn Phe Glu Arg Ser Ala Gly  
885 890 895

ctg cag gcg gga gtg gac gcg gta gag gac gcc agc agc atc gtg ttc 2736  
Leu Gln Ala Gly Val Asp Ala Val Glu Asp Ala Ser Ser Ile Val Phe  
900 905 910

ctc tgc gac ctg cac atc cac ttc cca ccc aac atc ctg gac ggc atc 2784  
Leu Cys Asp Leu His Ile His Phe Pro Pro Asn Ile Leu Asp Gly Ile  
915 920 925

cgc aag cac tgc gtg gag ggc agg ctg gcc ttc gcg ccc gtg gtc atg 2832  
Arg Lys His Cys Val Glu Gly Arg Leu Ala Phe Ala Pro Val Val Met  
930 935 940

cgc ctg agc tgc ggg agc tcg ccc cgg gac ccc cac ggt tac tgg gag 2880  
Arg Leu Ser Cys Gly Ser Ser Pro Arg Asp Pro His Gly Tyr Trp Glu  
945 950 955 960

gtg aac ggc ttt ggc ctt ttt ggg atc tac aag tcg gac ttt gac cgg 2928  
Val Asn Gly Phe Gly Leu Phe Gly Ile Tyr Lys Ser Asp Phe Asp Arg



965 970 975

gtt gga gga atg aac acg gag gag ttc cga gac cag tgg ggg ggt gaa 2976  
Val Gly Gly Met Asn Thr Glu Glu Phe Arg Asp Gln Trp Gly Gly Glu

980 985 990

gac tgg gag ctc ctg gac agg gtc ctg cag gca ggg ctg gag gtg gag 3024  
Asp Trp Glu Leu Leu Asp Arg Val Leu Gln Ala Gly Leu Glu Val Glu

995 1000 1005

cgg ctc cga ctg cgg aat ttc tat cac cac tac cac tcc aag agg ggc 3072  
Arg Leu Arg Leu Arg Asn Phe Tyr His His Tyr His Ser Lys Arg Gly

1010 1015 1020

atg tgg agc gtc cgc agc agg aag ggc tct cgc acg ggg gcg tct tga 3120  
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1025 1030 1035 1039

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<211> 998

<212> PRT

<213> Homo sapiens

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Met Gly Ser Pro Arg Ala Ala Arg Pro Pro Leu Leu Leu Arg Pro Val

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Lys Leu Leu Arg Arg Arg Phe Arg Leu Leu Leu Ala Leu Ala Val Val

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Ser Val Gly Leu Trp Thr Leu Tyr Leu Glu Leu Val Ala Ser Ala Gln  
35 40 45

Val Gly Gly Asn Pro Leu Asn Arg Arg Tyr Gly Ser Trp Arg Glu Leu  
50 55 60

Ala Lys Ala Leu Ala Ser Arg Asn Ile Pro Ala Val Asp Pro His Leu  
65 70 75 80

Gln Phe Tyr His Pro Gln Arg Leu Ser Leu Glu Asp His Asp Ile Asp  
85 90 95

Gln Gly Val Ser Ser Asn Ser Ser Tyr Leu Lys Trp Asn Lys Pro Val  
100 105 110

Pro Trp Leu Ser Glu Phe Arg Gly Arg Ala Asn Leu His Val Phe Glu  
115 120 125

Asp Trp Cys Gly Ser Ser Ile Gln Gln Leu Arg Arg Asn Leu His Phe  
130 135 140

Pro Leu Tyr Pro His Ile Arg Thr Thr Leu Arg Lys Leu Ala Val Ser  
145 150 155 160

Pro Lys Trp Thr Asn Tyr Gly Leu Arg Ile Phe Gly Tyr Leu His Pro  
165 170 175

Phe Thr Asp Gly Lys Ile Gln Phe Ala Ile Ala Ala Asp Asp Asn Ala  
180 185 190

Glu Phe Trp Leu Ser Leu Asp Asp Gln Val Ser Gly Leu Gln Leu Leu  
195 200 205

Ala Ser Val Gly Lys Thr Gly Lys Glu Trp Thr Ala Pro Gly Glu Phe  
210 215 220

Gly Lys Phe Arg Ser Gln Ile Ser Lys Pro Val Ser Leu Ser Ala Ser  
225 230 235 240

His Arg Tyr Tyr Phe Glu Val Leu His Lys Gln Asn Glu Glu Gly Thr  
245 250 255

Asp His Val Glu Val Ala Trp Arg Arg Asn Asp Pro Gly Ala Lys Phe  
260 265 270

Thr Ile Ile Asp Ser Leu Ser Leu Ser Leu Phe Thr Asn Glu Thr Phe  
275 280 285

Leu Gln Met Asp Glu Val Gly His Ile Pro Gln Thr Ala Ala Ser His  
290 295 300

Val Asp Ser Ser Asn Ala Leu Pro Arg Asp Glu Gln Pro Pro Ala Asp  
305 310 315 320

Met Leu Arg Pro Asp Pro Arg Asp Thr Leu Tyr Arg Val Pro Leu Ile  
325 330 335

Pro Lys Ser His Leu Arg His Val Leu Pro Asp Cys Pro Tyr Lys Pro  
340 345 350

Ser Tyr Leu Val Asp Gly Leu Pro Leu Gln Arg Tyr Gln Gly Leu Arg  
355 360 365

Phe Val His Leu Ser Phe Val Tyr Pro Asn Asp Tyr Thr Arg Leu Ser  
370 375 380

His Met Glu Thr His Asn Lys Cys Phe Tyr Gln Glu Asn Ala Tyr Tyr  
385 390 395 400

Gln Asp Arg Phe Ser Phe Gln Glu Tyr Ile Arg Ile Asp Gln Pro Glu  
405 410 415

Lys Gln Gly Leu Glu Gln Pro Gly Phe Glu Glu Asn Leu Leu Glu Glu  
420 425 430

Ser Gln Tyr Gly Glu Val Ala Glu Glu Thr Pro Ala Ser Asn Asn Gln  
435 440 445

Asn Ala Arg Met Leu Glu Gly Arg Gln Thr Pro Ala Ser Thr Leu Glu  
450 455 460

Gln Asp Ala Thr Asp Tyr Arg Leu Arg Ser Leu Arg Lys Leu Leu Ala  
465 470 475 480

Gln Pro Arg Glu Gly Leu Leu Ala Pro Phe Ser Lys Arg Asn Ser Thr

485

490

495

Ala Ser Phe Pro Gly Arg Thr Ser His Ile Pro Val Gln Gln Pro Glu

500

505

510

Lys Arg Lys Gln Lys Pro Ser Pro Glu Pro Ser Gln Asp Ser Pro His

515

520

525

Ser Asp Lys Trp Pro Pro Gly His Pro Val Lys Asn Leu Pro Gln Met

530

535

540

Arg Gly Pro Arg Pro Arg Pro Ala Gly Asp Ser Pro Arg Lys Thr Gln

545

550

555

560

Trp Leu Asn Gln Val Glu Ser Tyr Ile Ala Glu Gln Arg Arg Gly Asp

565

570

575

Arg Met Arg Pro Gln Ala Pro Gly Arg Gly Trp His Gly Glu Glu Glu

580

585

590

Val Val Ala Ala Ala Gly Gln Glu Gly Gln Val Glu Gly Glu Glu Glu

595

600

605

Gly Glu Glu Glu Glu Glu Glu Glu Asp Met Ser Glu Val Phe Glu Tyr

610

615

620

Val Pro Val Phe Asp Pro Val Val Asn Trp Asp Gln Thr Phe Ser Ala

625

630

635

640

Arg Asn Leu Asp Phe Gln Ala Leu Arg Thr Asp Trp Ile Asp Leu Ser  
645 650 655

Cys Asn Thr Ser Gly Asn Leu Leu Leu Pro Glu Gln Glu Ala Leu Glu  
660 665 670

Val Thr Arg Val Phe Leu Lys Lys Leu Asn Gln Arg Ser Arg Gly Arg  
675 680 685

Tyr Gln Leu Gln Arg Ile Val Asn Val Glu Lys Arg Gln Asp Gln Leu  
690 695 700

Arg Gly Gly Arg Tyr Leu Leu Glu Leu Glu Leu Leu Glu Gln Gly Gln  
705 710 715 720

Arg Val Val Arg Leu Ser Glu Tyr Val Ser Ala Arg Gly Trp Gln Gly  
725 730 735

Ile Asp Pro Ala Gly Gly Glu Glu Val Glu Ala Arg Asn Leu Gln Gly  
740 745 750

Leu Val Trp Asp Pro His Asn Arg Arg Arg Gln Val Leu Asn Thr Arg  
755 760 765

Ala Gln Glu Pro Lys Leu Cys Trp Pro Gln Gly Phe Ser Trp Ser His  
770 775 780

Arg Ala Val Val His Phe Val Val Pro Val Lys Asn Gln Ala Arg Trp  
785 790 795 800

Val Gln Gln Phe Ile Lys Asp Met Glu Asn Leu Phe Gln Val Thr Gly

805

810

815

Asp Pro His Phe Asn Ile Val Ile Thr Asp Tyr Ser Ser Glu Asp Met

820

825

830

Asp Val Glu Met Ala Leu Lys Arg Ser Lys Leu Arg Ser Tyr Gln Tyr

835

840

845

Val Lys Leu Ser Gly Asn Phe Glu Arg Ser Ala Gly Leu Gln Ala Gly

850

855

860

Ile Asp Leu Val Lys Asp Pro His Ser Ile Ile Phe Leu Cys Asp Leu

865

870

875

880

His Ile His Phe Pro Ala Gly Val Ile Asp Ala Ile Arg Lys His Cys

885

890

895

Val Glu Gly Lys Met Ala Phe Ala Pro Met Val Met Arg Leu His Cys

900

905

910

Gly Ala Thr Pro Gln Trp Pro Glu Gly Tyr Trp Glu Val Asn Gly Phe

915

920

925

Gly Leu Leu Gly Ile Tyr Lys Ser Asp Leu Asp Arg Ile Gly Gly Met

930

935

940

Asn Thr Lys Glu Phe Arg Asp Arg Trp Gly Gly Glu Asp Trp Glu Leu

945                                      950                                      955                                      960

Leu Asp Arg Ile Leu Gln Ala Gly Leu Asp Val Glu Arg Leu Ser Leu

965                                      970                                      975

Arg Asn Phe Phe His His Phe His Ser Lys Arg Gly Met Trp Ser Arg

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Arg Gln Met Lys Thr Leu

995                                      998

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<400> 4

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1                                      5                                      10                                      15

aag ctg ctg cgg agg cgc ttc cgg ctg ctg ctg gcg ctc gcc gtg gtg 96

Lys Leu Leu Arg Arg Arg Phe Arg Leu Leu Leu Ala Leu Ala Val Val

20                                      25                                      30

tct gtg ggg ctc tgg act ctg tat ctg gaa ctg gtg gcg tcg gcc cag 144

Ser Val Gly Leu Trp Thr Leu Tyr Leu Glu Leu Val Ala Ser Ala Gln

35                                      40                                      45



gtc ggc ggg aac ccc ctg aac cgg agg tac ggc agc tgg aga gaa cta 192  
Val Gly Gly Asn Pro Leu Asn Arg Arg Tyr Gly Ser Trp Arg Glu Leu  
50 55 60

gcc aag gct ctg gcc agc agg aac att cca gct gtg gat cca cac ctc 240  
Ala Lys Ala Leu Ala Ser Arg Asn Ile Pro Ala Val Asp Pro His Leu  
65 70 75 80

cag ttc tac cat ccc cag agg ctg agc ctc gag gac cac gac att gac 288  
Gln Phe Tyr His Pro Gln Arg Leu Ser Leu Glu Asp His Asp Ile Asp  
85 90 95

caa ggg gtg agc agt aac agc agc tac ttg aag tgg aac aag cct gtc 336  
Gln Gly Val Ser Ser Asn Ser Ser Tyr Leu Lys Trp Asn Lys Pro Val  
100 105 110

ccc tgg ctc tca gag ttc cgg ggc cgt gcc aac ctg cat gtg ttt gaa 384  
Pro Trp Leu Ser Glu Phe Arg Gly Arg Ala Asn Leu His Val Phe Glu  
115 120 125

gac tgg tgt ggc agc tct atc cag cag ctc agg agg aac ctg cat ttc 432  
Asp Trp Cys Gly Ser Ser Ile Gln Gln Leu Arg Arg Asn Leu His Phe  
130 135 140

cca ctg tac ccc cat att cgc aca acc ctg agg aag ctt gct gtg tcc 480  
Pro Leu Tyr Pro His Ile Arg Thr Thr Leu Arg Lys Leu Ala Val Ser  
145 150 155 160

ccc aaa tgg acc aac tat ggc ctc cgc atc ttt ggc tac ctg cac ccc 528  
Pro Lys Trp Thr Asn Tyr Gly Leu Arg Ile Phe Gly Tyr Leu His Pro  
165 170 175

ttt act gat ggg aaa atc cag ttt gcc att gct gca gat gac aac gcg 576  
Phe Thr Asp Gly Lys Ile Gln Phe Ala Ile Ala Ala Asp Asp Asn Ala  
180 185 190

gag ttc tgg ctg agc ctc gat gac cag gtc tca ggc ctc cag ctg ctg 624  
Glu Phe Trp Leu Ser Leu Asp Asp Gln Val Ser Gly Leu Gln Leu Leu  
195 200 205

gcc agt gtg ggc aag act gga aag gag tgg acc gcc ccg gga gag ttt 672  
Ala Ser Val Gly Lys Thr Gly Lys Glu Trp Thr Ala Pro Gly Glu Phe  
210 215 220

ggg aaa ttt cgg agc caa att tcc aag ccg gtg agc ctg tca gcc tcc 720  
Gly Lys Phe Arg Ser Gln Ile Ser Lys Pro Val Ser Leu Ser Ala Ser  
225 230 235 240

cac agg tac tac ttc gag gtg ctg cac aag cag aat gag gag ggc acc 768  
His Arg Tyr Tyr Phe Glu Val Leu His Lys Gln Asn Glu Glu Gly Thr  
245 250 255

gac cac gtg gaa gtt gca tgg cga cgg aac gac cct gga gcc aag ttc 816  
Asp His Val Glu Val Ala Trp Arg Arg Asn Asp Pro Gly Ala Lys Phe  
260 265 270

acc atc att gac tcc ctc tcc ctg tcc ctc ttc aca aat gag acg ttc 864

Thr Ile Ile Asp Ser Leu Ser Leu Ser Leu Phe Thr Asn Glu Thr Phe

275

280

285

cta cag atg gat gag gtg ggc cac atc cca cag aca gca gcc agc cac 912

Leu Gln Met Asp Glu Val Gly His Ile Pro Gln Thr Ala Ala Ser His

290

295

300

gtg gac tcc tcc aac gct ctt ccc agg gat gag cag ccg ccc gct gac 960

Val Asp Ser Ser Asn Ala Leu Pro Arg Asp Glu Gln Pro Pro Ala Asp

305

310

315

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atg ctt cgg cct gac ccc cgg gac acc ctc tat cga gtg cct ctg atc 1008

Met Leu Arg Pro Asp Pro Arg Asp Thr Leu Tyr Arg Val Pro Leu Ile

325

330

335

ccc aag tcg cat ctc cgc cac gtc ctg cct gac tgt ccc tac aaa ccc 1056

Pro Lys Ser His Leu Arg His Val Leu Pro Asp Cys Pro Tyr Lys Pro

340

345

350

agc tat ctg gtg gat ggg ctt cct ctg cag cgc tac cag gga ctc cgg 1104

Ser Tyr Leu Val Asp Gly Leu Pro Leu Gln Arg Tyr Gln Gly Leu Arg

355

360

365

ttt gtt cat ctg tct ttt gtt tac ccc aat gac tat acc cgc ctg agc 1152

Phe Val His Leu Ser Phe Val Tyr Pro Asn Asp Tyr Thr Arg Leu Ser

370

375

380

cac atg gag acc cac aat aaa tgt ttc tac cag gaa aac gcc tac tac 1200

His Met Glu Thr His Asn Lys Cys Phe Tyr Gln Glu Asn Ala Tyr Tyr

385	390	395	400	
caa gac cgg ttc agc ttt cag gag tac atc agg att gac cag cct gag				1248
Gln Asp Arg Phe Ser Phe Gln Glu Tyr Ile Arg Ile Asp Gln Pro Glu				
	405	410	415	
aag cag ggg ctg gag cag cca ggt ttt gag gaa aac ctt cta gaa gag				1296
Lys Gln Gly Leu Glu Gln Pro Gly Phe Glu Glu Asn Leu Leu Glu Glu				
	420	425	430	
tcc cag tat ggg gaa gtg gca gag gag acc cct gcc tcc aac aac cag				1344
Ser Gln Tyr Gly Glu Val Ala Glu Glu Thr Pro Ala Ser Asn Asn Gln				
	435	440	445	
aat gcc agg atg ctt gag gga aga cag aca cct gcc tcc acc ctg gag				1392
Asn Ala Arg Met Leu Glu Gly Arg Gln Thr Pro Ala Ser Thr Leu Glu				
	450	455	460	
caa gat gcc act gac tac cgc ctc cga agc ctg cgg aaa ctc ctg gct				1440
Gln Asp Ala Thr Asp Tyr Arg Leu Arg Ser Leu Arg Lys Leu Leu Ala				
	465	470	475	480
cag ccc cgg gag ggc ctg ctg gcc ccc ttc tcc aag cgg aac tcc aca				1488
Gln Pro Arg Glu Gly Leu Leu Ala Pro Phe Ser Lys Arg Asn Ser Thr				
	485	490	495	
gcg tcc ttc cca ggg agg acc agc cac att cca gtg cag cag cca gag				1536
Ala Ser Phe Pro Gly Arg Thr Ser His Ile Pro Val Gln Gln Pro Glu				
	500	505	510	

aag agg aag caa aaa ccc agc cct gag ccc agc caa gat tca cct cat 1584

Lys Arg Lys Gln Lys Pro Ser Pro Glu Pro Ser Gln Asp Ser Pro His

515

520

525

tcc gac aag tgg cct cct ggg cac cct gtg aag aac ctg cct cag atg 1632

Ser Asp Lys Trp Pro Pro Gly His Pro Val Lys Asn Leu Pro Gln Met

530

535

540

agg ggg ccc agg ccc agg ccc gct ggt gac agc ccc agg aag act cag 1680

Arg Gly Pro Arg Pro Arg Pro Ala Gly Asp Ser Pro Arg Lys Thr Gln

545

550

555

560

tgg ctg aac cag gtg gag tcg tac atc gca gag cag aga cgg ggt gac 1728

Trp Leu Asn Gln Val Glu Ser Tyr Ile Ala Glu Gln Arg Arg Gly Asp

565

570

575

agg atg cgg cct cag gcc ccc gga agg ggc tgg cat ggg gag gag gaa 1776

Arg Met Arg Pro Gln Ala Pro Gly Arg Gly Trp His Gly Glu Glu Glu

580

585

590

gtg gtg gcg gcc gca ggc cag gaa gga caa gtg gag gga gag gaa gag 1824

Val Val Ala Ala Ala Gly Gln Glu Gly Gln Val Glu Gly Glu Glu Glu

595

600

605

ggg gaa gaa gag gag gag gaa gag gat atg agt gag gtg ttc gag tac 1872

Gly Glu Glu Glu Glu Glu Glu Glu Asp Met Ser Glu Val Phe Glu Tyr

610

615

620

gta cct gig tit gac ccg gta gta aac tgg gac cag acc ttc agt gcc 1920  
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625 630 635 640

cgg aat ctc gac ttc caa gcc ctg agg act gac tgg atc gat ctg agc 1968  
Arg Asn Leu Asp Phe Gln Ala Leu Arg Thr Asp Trp Ile Asp Leu Ser  
645 650 655

tgt aac aca tct ggc aac ctg ctg ctt cca gag cag gaa gct ctg gag 2016  
Cys Asn Thr Ser Gly Asn Leu Leu Leu Pro Glu Gln Glu Ala Leu Glu  
660 665 670

gtc acg cga gtc ttc ttg aag aag ctc aac cag agg agc cgg ggg agg 2064  
Val Thr Arg Val Phe Leu Lys Lys Leu Asn Gln Arg Ser Arg Gly Arg  
675 680 685

tac cag cta cag cgc att gtg aac gtg gaa aag cgt cag gac cag cta 2112  
Tyr Gln Leu Gln Arg Ile Val Asn Val Glu Lys Arg Gln Asp Gln Leu  
690 695 700

cgt ggg ggt cgc tac ctc ctg gag ctt gaa ctg ttg gaa caa ggc cag 2160  
Arg Gly Gly Arg Tyr Leu Leu Glu Leu Glu Leu Leu Glu Gln Gly Gln  
705 710 715 720

cgc gtg gtg cgg ctc tcg gag tat gtg tct gca cga ggc tgg cag ggc 2208  
Arg Val Val Arg Leu Ser Glu Tyr Val Ser Ala Arg Gly Trp Gln Gly  
725 730 735

atc gat cca gct ggt ggg gag gag gtc gag gcc cgg aac ctg caa ggc 2256

Ile Asp Pro Ala Gly Gly Glu Glu Val Glu Ala Arg Asn Leu Gln Gly  
740 745 750

ctg gtc tgg gac cca cac aac cgt agg aga cag gtc ctg aat acc cgg 2304  
Leu Val Trp Asp Pro His Asn Arg Arg Arg Gln Val Leu Asn Thr Arg  
755 760 765

gcc caa gag ccc aag ctg tgc tgg cct cag ggt ttc tcc tgg agt cac 2352  
Ala Gln Glu Pro Lys Leu Cys Trp Pro Gln Gly Phe Ser Trp Ser His  
770 775 780

cga gcc gtg gtc cac ttc gtc gtg cct gtg aag aac cag gca cgc tgg 2400  
Arg Ala Val Val His Phe Val Val Pro Val Lys Asn Gln Ala Arg Trp  
785 790 795 800

gta cag caa ttc atc aaa gac atg gaa aac ctg ttc cag gtc acc ggt 2448  
Val Gln Gln Phe Ile Lys Asp Met Glu Asn Leu Phe Gln Val Thr Gly  
805 810 815

gac cca cac ttc aac atc gtc atc act gac tat agc agt gag gac atg 2496  
Asp Pro His Phe Asn Ile Val Ile Thr Asp Tyr Ser Ser Glu Asp Met  
820 825 830

gat gtt gag atg gca ctg aag agg tcc aag ctg cgg agc tac cag tac 2544  
Asp Val Glu Met Ala Leu Lys Arg Ser Lys Leu Arg Ser Tyr Gln Tyr  
835 840 845

gtg aag cta agt gga aac ttt gaa cgc tca gct gga ctt cag gct ggc 2592  
Val Lys Leu Ser Gly Asn Phe Glu Arg Ser Ala Gly Leu Gln Ala Gly

850 855 860  
ata gac ctc gtg aag gac ccg cac agc atc atc ttc ctc tgt gac ctc 2640  
Ile Asp Leu Val Lys Asp Pro His Ser Ile Ile Phe Leu Cys Asp Leu  
865 870 875 880  
cac atc cac ttc cca gct gga gtc atc gat gcc att cgg aag cac tgt 2688  
His Ile His Phe Pro Ala Gly Val Ile Asp Ala Ile Arg Lys His Cys  
885 890 895  
gtg gag gga aag atg gcc ttt gcc ccc atg gtg atg agg ctg cat tgt 2736  
Val Glu Gly Lys Met Ala Phe Ala Pro Met Val Met Arg Leu His Cys  
900 905 910  
ggg gcc acc ccc cag tgg cct gag ggc tac tgg gag gtg aat ggg ttc 2784  
Gly Ala Thr Pro Gln Trp Pro Glu Gly Tyr Trp Glu Val Asn Gly Phe  
915 920 925  
ggg ctg ctt ggc atc tac aag tct gac ctg gac agg att ggg ggc atg 2832  
Gly Leu Leu Gly Ile Tyr Lys Ser Asp Leu Asp Arg Ile Gly Gly Met  
930 935 940  
aac acc aag gag ttc cga gac cgc tgg ggc ggg gaa gac tgg gag ctg 2880  
Asn Thr Lys Glu Phe Arg Asp Arg Trp Gly Gly Glu Asp Trp Glu Leu  
945 950 955 960  
ctg gac agg ata ctc caa gcg ggc ctg gac gtg gag cgt ctc tcc ctc 2928  
Leu Asp Arg Ile Leu Gln Ala Gly Leu Asp Val Glu Arg Leu Ser Leu  
965 970 975



agg aat ttc ttc cat cat ttc cat tcc aag cga ggc atg tgg agc cgt 2976

Arg Asn Phe Phe His His Phe His Ser Lys Arg Gly Met Trp Ser Arg

980

985

990

cgc cag atg aag acg ctg tag

2997

Arg Gln Met Lys Thr Leu

995

998

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<400> 6

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<400> 14

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<210> 15

<211> 24

<212> DNA

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<223> Oligonucleotide primer used in PCR for cloning GalNAc-T2 cDNA

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<223> Oligonucleotide primer used in PCR for cloning GalNAc-T2 cDNA

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ggaattcgag gtacggcagc tggagagaa 29

<210> 19

<211> 32

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide primer used in PCR for cloning GalNAc-T2 cDNA

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acgcgtcgac ctacagcgtc ttcatctggc ga

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ctggtggatt tcgagagcga 20

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<210> 22

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<213> Artificial Sequence

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<223> Oligonucleotide probe used in PCR for detecting GalNAc-T1 cDNA

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<210> 23

<211> 26

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<223> Oligonucleotide primer used in PCR for amplifying GalNAc-T2 cDNA

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<212> DNA

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<223> Oligonucleotide probe used in PCR for detecting GalNAc-T2 cDNA

<400> 25

ctcgtgaagg acccgca 17

&lt;210&gt; 26

&lt;211&gt; 1034

&lt;212&gt; PRT

&lt;213&gt; Mouse

&lt;400&gt; 26

Met Pro Trp Phe Pro Val Lys Lys Val Arg Lys Gln Met Lys Leu Leu

1 5 10 15

Leu Leu Leu Leu Leu Leu Thr Cys Ala Ala Trp Leu Thr Tyr Val His

20 25 30

Arg Ser Leu Val Arg Pro Gly Arg Ala Leu Arg Gln Arg Leu Gly Tyr

35 40 45

Gly Arg Asp Gly Glu Lys Leu Thr Gly Val Thr Asp Ser Arg Gly Val

50 55 60

Arg Val Pro Ser Ser Thr Gln Arg Ser Glu Asp Ser Ser Glu Ser His

65 70 75 80

Glu Glu Glu Gln Ala Pro Glu Gly Arg Gly Pro Asn Met Leu Phe Pro

85 90 95

Gly Gly Pro Arg Lys Pro Pro Pro Leu Asn Leu Thr His Gln Thr Pro

100 105 110

Pro Trp Arg Glu Glu Phe Lys Gly Gln Val Asn Leu His Val Phe Glu

115	120	125
Asp Trp Cys Gly Gly Ala Val Gly His Leu Arg Arg Asn Leu His Phe		
130	135	140
Pro Leu Phe Pro His Thr Arg Thr Thr Val Thr Lys Leu Ala Val Ser		
145	150	155
160		
Pro Lys Trp Lys Asn Tyr Gly Leu Arg Ile Phe Gly Phe Ile His Pro		
165	170	175
Ala Arg Asp Gly Asp Ile Gln Phe Ser Val Ala Ser Asp Asp Asn Ser		
180	185	190
Glu Phe Trp Leu Ser Leu Asp Glu Ser Pro Ala Ala Ala Gln Leu Val		
195	200	205
Ala Phe Val Gly Lys Thr Gly Ser Glu Trp Thr Ala Pro Gly Glu Phe		
210	215	220
Thr Lys Phe Ser Ser Gln Val Ser Lys Pro Arg Arg Leu Met Ala Ser		
225	230	235
240		
Arg Arg Tyr Tyr Phe Glu Leu Leu His Lys Gln Asp Asp Lys Gly Ser		
245	250	255
Asp His Val Glu Val Gly Trp Arg Ala Phe Leu Pro Gly Leu Lys Phe		
260	265	270

Glu Ile Ile Asp Ser Ala His Ile Ser Leu Tyr Thr Asp Glu Ser Ser  
275 280 285

Leu Lys Met Asp His Val Ala His Val Pro Gln Ser Pro Ala Ser His  
290 195 300

Ile Gly Gly Phe Pro Pro Gln Gly Glu Pro Ser Ala Asp Met Leu His  
305 310 315 320

Pro Asp Pro Arg Asp Thr Phe Phe Leu Thr Pro Arg Met Glu Pro Leu  
325 330 335

Ser Leu Glu Asn Val Leu Glu Pro Cys Ala Tyr Ala Pro Thr Tyr Ile  
340 345 350

Leu Lys Asp Phe Pro Ile Ala Arg Tyr Gln Gly Leu Gln Phe Val Tyr  
355 360 365

Leu Ser Phe Ile Tyr Pro Asn Asp His Thr Arg Leu Thr His Met Glu  
370 375 380

Thr Asp Asn Lys Cys Phe Tyr Arg Glu Ser Pro Leu Tyr Leu Glu Arg  
385 390 395 400

Phe Gly Phe Tyr Lys Tyr Met Lys Met Asp Lys Glu Glu Gly Glu Glu  
405 410 415

Asp Glu Glu Glu Glu Val Gln Arg Arg Ala Phe Leu Phe Leu Asn Pro  
420 425 430

Asp Asp Phe Leu Asp Glu Glu Asp Glu Gln Asp Leu Leu Asp Ser Leu

435

440

445

Glu Pro Thr Asp Ala Ser Val Gln Gln Ser His Arg Thr Pro Thr Pro

450

455

460

Ala Ala Ser Thr Gly Thr Thr Ala Ser Pro Thr Pro Pro Thr Thr Ser

465

470

475

480

Pro Leu Asp Glu Gln Thr Leu Arg His Ser Arg Ala Leu Asn Trp Ala

485

490

495

Pro Arg Pro Leu Pro Leu Phe Leu Gly Arg Ala Pro Pro Pro Arg Thr

500

505

510

Val Glu Lys Ser Pro Ser Lys Val Tyr Val Thr Arg Val Arg Pro Gly

515

520

525

Gln Arg Ala Ser Pro Arg Ala Leu Arg Asp Ser Pro Trp Pro Pro Phe

530

535

540

Pro Gly Val Phe Leu Arg Pro Lys Pro Leu Pro Arg Val Gln Leu Arg

545

550

555

560

Val Pro Pro His Pro Pro Arg Thr Gln Gly Tyr Arg Thr Ser Gly Pro

565

570

575

Lys Val Thr Glu Leu Lys Pro Pro Val Arg Ala Gln Thr Ser Gln Gly

580

585

590

Gly Arg Glu Gly Gln Leu His Gly Gln Gly Leu Met Val Pro Thr Val

595

600

605

Asp Leu Asn Ser Ser Val Glu Thr Gln Pro Val Thr Ser Phe Leu Ser

610

615

620

Leu Ser Gln Val Ser Arg Pro Gln Leu Pro Gly Glu Gly Glu Glu Gly

625

630

635

640

Glu Glu Asp Gly Ala Pro Gly Asp Glu Ala Thr Ser Glu Asp Ser Glu

645

650

655

Glu Glu Glu Glu Pro Ala Ala Gly Arg Pro Leu Gly Arg Trp Arg Glu

660

665

670

Asp Ala Ile Asn Trp Gln Arg Thr Phe Ser Val Gly Ala Met Asp Phe

675

680

685

Glu Leu Leu Arg Ser Asp Trp Asn Asp Leu Arg Cys Asn Val Ser Gly

690

695

700

Asn Leu Gln Leu Pro Glu Ala Glu Ala Val Asp Val Val Ala Gln Tyr

705

710

715

720

Met Glu Arg Leu Asn Ala Lys His Gly Gly Arg Phe Ser Leu Leu Arg

725

730

735

Ile Val Asn Val Glu Lys Arg Arg Asp Ser Ala Arg Gly Ser Arg Phe  
740 745 750

Leu Leu Glu Leu Glu Leu Gln Glu Arg Gly Gly Ser Arg Gln Arg Leu  
755 760 765

Ser Glu Tyr Val Phe Leu Arg Leu Pro Gly Ala Arg Val Gly Asp Glu  
770 775 780

Asp Gly Glu Ser Pro Glu Pro Pro Pro Ala Ala Ser Ile His Pro Asp  
785 790 795 800

Ser Arg Pro Glu Leu Cys Arg Pro Leu His Leu Ala Trp Arg Gln Asp  
805 810 815

Val Met Val His Phe Ile Val Pro Val Lys Asn Gln Ala Arg Trp Val  
820 825 830

Val Gln Phe Leu Ala Asp Met Thr Ala Leu His Val His Thr Gly Asp  
835 840 845

Ser Tyr Phe Asn Ile Ile Leu Val Asp Phe Glu Ser Glu Asp Met Asp  
850 855 860

Val Glu Arg Ala Leu Arg Ala Ala Gln Leu Pro Arg Tyr Gln Tyr Leu  
865 870 875 880

Lys Arg Thr Gly Asn Phe Glu Arg Ser Ala Gly Leu Gln Thr Gly Val  
885 890 895

Asp Ala Val Glu Asp Pro Ser Ser Ile Val Phe Leu Cys Asp Leu His  
900 905 910

Ile His Phe Pro Pro Asn Ile Leu Asp Ser Ile Arg Lys His Cys Val  
915 920 925

Glu Gly Lys Leu Ala Phe Ala Pro Val Val Met Arg Leu Gly Cys Gly  
930 935 940

Ser Ser Pro Trp Asp Pro His Gly Tyr Trp Glu Val Asn Gly Phe Gly  
945 950 955 960

Leu Phe Gly Ile Tyr Lys Ser Asp Phe Asp Arg Val Gly Gly Met Asn  
965 970 975

Thr Glu Glu Phe Arg Asp Gln Trp Gly Gly Glu Asp Trp Glu Leu Leu  
980 985 990

Asp Arg Val Leu Gln Ala Gly Leu Glu Val Glu Arg Leu Arg Leu Arg  
995 1000 1005

His Phe Tyr His His Tyr His Ser Lys Arg Gly Met Trp Ala Thr Arg  
1010 1015 1020

Ser Arg Lys Gly Ala Arg Ala Gln Arg Ser  
1025 1030



&lt;210&gt; 27

&lt;211&gt; 3105

&lt;212&gt; DNA

&lt;213&gt; Mouse

&lt;400&gt; 27

atg ccg tgg ttc ccg gtg aag aag gtc cgc aag cag atg aag ctg ctg 48  
Met Pro Trp Phe Pro Val Lys Lys Val Arg Lys Gln Met Lys Leu Leu  
1 5 10 15

ctg ctg ttg ctg ctg ctc acc tgc gcc gcg tgg ctc acg tat gtg cac 96  
Leu Leu Leu Leu Leu Leu Thr Cys Ala Ala Trp Leu Thr Tyr Val His  
20 25 30

cgg agc ctg gtg cgc ccg ggc cgc gcg cta cgc cag cgg ctg ggc tac 144  
Arg Ser Leu Val Arg Pro Gly Arg Ala Leu Arg Gln Arg Leu Gly Tyr  
35 40 45

ggg cga gat ggg gag aag ctg acc ggt gtg acc gat agc cgc gga gtc 192  
Gly Arg Asp Gly Glu Lys Leu Thr Gly Val Thr Asp Ser Arg Gly Val  
50 55 60

cga gtg cca tcg tcc aca cag agg tcg gag gac tcg agt gaa agt cat 240  
Arg Val Pro Ser Ser Thr Gln Arg Ser Glu Asp Ser Ser Glu Ser His  
65 70 75 80

gaa gag gag cag gcg ccc gag ggg cgg ggc cca aac atg ctg ttt cct 288  
Glu Glu Glu Gln Ala Pro Glu Gly Arg Gly Pro Asn Met Leu Phe Pro  
85 90 95

gga gga cct agg aag cca ccc cca ctg aac ctc acc cac cag aca ccc 336  
Gly Gly Pro Arg Lys Pro Pro Pro Leu Asn Leu Thr His Gln Thr Pro  
100 105 110

cca tgg cgg gaa gag ttc aaa gga cag gtg aac ctg cac gtg ttt gag 384  
Pro Trp Arg Glu Glu Phe Lys Gly Gln Val Asn Leu His Val Phe Glu  
115 120 125

gac tgg tgt gga ggt gct gtg ggc cac ctg aga cgg aat ctg cac ttc 432  
Asp Trp Cys Gly Gly Ala Val Gly His Leu Arg Arg Asn Leu His Phe  
130 135 140

cca ctc ttt cct cac act cgt act acg gtg aca aag tta gct gtg tcc 480  
Pro Leu Phe Pro His Thr Arg Thr Thr Val Thr Lys Leu Ala Val Ser  
145 150 155 160

cct aag tgg aag aac tat gga ctc cgg att ttt ggc ttc atc cac cca 528  
Pro Lys Trp Lys Asn Tyr Gly Leu Arg Ile Phe Gly Phe Ile His Pro  
165 170 175

gcc aga gat gga gac atc cag ttc tct gtg gct tcg gat gac aac tct 576  
Ala Arg Asp Gly Asp Ile Gln Phe Ser Val Ala Ser Asp Asp Asn Ser  
180 185 190

gag ttc tgg ctg agt ttg gat gag agc cca gca gcc gcc cag ctt gta 624  
Glu Phe Trp Leu Ser Leu Asp Glu Ser Pro Ala Ala Ala Gln Leu Val  
195 200 205

gcc ttt gtg ggc aag act ggc tcc gag tgg acc gca cct gga gaa ttc 672  
Ala Phe Val Gly Lys Thr Gly Ser Glu Trp Thr Ala Pro Gly Glu Phe  
210 215 220

acc aag ttc agc tcc cag gtg tct aag cca cgt cgg ctc atg gcc tcc 720  
Thr Lys Phe Ser Ser Gln Val Ser Lys Pro Arg Arg Leu Met Ala Ser  
225 230 235 240

cgg aga tac tac ttt gaa ctg ctc cac aag caa gat gac aag ggt tca 768  
Arg Arg Tyr Tyr Phe Glu Leu Leu His Lys Gln Asp Asp Lys Gly Ser  
245 250 255

gac cat gtg gaa gtg ggt tgg cga gct ttc ctg cct ggt ctg aag ttc 816  
Asp His Val Glu Val Gly Trp Arg Ala Phe Leu Pro Gly Leu Lys Phe  
260 265 270

gag atc att gat tct gct cac att tcc ctg tac aca gat gag tca tct 864  
Glu Ile Ile Asp Ser Ala His Ile Ser Leu Tyr Thr Asp Glu Ser Ser  
275 280 285

ctg aag atg gac cat gtg gcc cat gtg cct cag tct cca gcc agc cac 912  
Leu Lys Met Asp His Val Ala His Val Pro Gln Ser Pro Ala Ser His  
290 195 300

ata gga gga ttc ccg ccg cag ggg gaa ccc agc gcc gac atg ctg cac 960  
Ile Gly Gly Phe Pro Pro Gln Gly Glu Pro Ser Ala Asp Met Leu His  
305 310 315 320

cca gac ccc agg gat acc ttc ttc ctc act cct cgg atg gaa cct ttg 1008

Pro Asp Pro Arg Asp Thr Phe Phe Leu Thr Pro Arg Met Glu Pro Leu	
325	335
agc ctg gag aat gtt ctg gag ccc tgt gcc tat gcc ccc acc tat atc	1056
Ser Leu Glu Asn Val Leu Glu Pro Cys Ala Tyr Ala Pro Thr Tyr Ile	
340	350
ctc aag gat ttc ccc ata gcc aga tac caa gga cta cag ttt gig tac	1104
Leu Lys Asp Phe Pro Ile Ala Arg Tyr Gln Gly Leu Gln Phe Val Tyr	
355	365
ctg tcc ttc atc tac ccc aat gac cat acc cgt ctc act cac atg gag	1152
Leu Ser Phe Ile Tyr Pro Asn Asp His Thr Arg Leu Thr His Met Glu	
370	380
aca gac aac aag tgc ttc tac cgt gag tcc cca cta tac ctg gaa agg	1200
Thr Asp Asn Lys Cys Phe Tyr Arg Glu Ser Pro Leu Tyr Leu Glu Arg	
385	395
400	
ttt ggg ttc tat aaa tac atg aaa atg gac aag gag gag gga gag gaa	1248
Phe Gly Phe Tyr Lys Tyr Met Lys Met Asp Lys Glu Glu Gly Glu Glu	
405	415
gat gag gag gaa gaa gtt cag cgt aga gcc ttc ctc ttc ctc aac cca	1296
Asp Glu Glu Glu Glu Val Gln Arg Arg Ala Phe Leu Phe Leu Asn Pro	
420	430
gat gac ttc ctg gat gag gag gat gag cag gat ctg tta gac agc ctg	1344
Asp Asp Phe Leu Asp Glu Glu Asp Glu Gln Asp Leu Leu Asp Ser Leu	

435	440	445	
gag ccc acc gat gca tct gta cag cag agc cac agg acc ccc acc cca			1392
Glu Pro Thr Asp Ala Ser Val Gln Gln Ser His Arg Thr Pro Thr Pro			
450	455	460	
gca gcc tcc act gga acg aca gcc agc ccg acc cca cct aca act agt			1440
Ala Ala Ser Thr Gly Thr Thr Ala Ser Pro Thr Pro Pro Thr Thr Ser			
465	470	475	480
cct ctg gac gag cag acc ctc aga cac tcc cgg gca ctg aat tgg gcc			1488
Pro Leu Asp Glu Gln Thr Leu Arg His Ser Arg Ala Leu Asn Trp Ala			
485	490	495	
cca cgc ccc ctg ccc ctc ttc ttg ggg cga gct cca cct ccc cga act			1536
Pro Arg Pro Leu Pro Leu Phe Leu Gly Arg Ala Pro Pro Pro Arg Thr			
500	505	510	
gtg gag aag tcg cct tca aag gtg tac gtg acc agg gtc cga cct gga			1584
Val Glu Lys Ser Pro Ser Lys Val Tyr Val Thr Arg Val Arg Pro Gly			
515	520	525	
cag cgg gct tcc ccg agg gca ttg cga gac tca ccc tgg cca ccc ttc			1632
Gln Arg Ala Ser Pro Arg Ala Leu Arg Asp Ser Pro Trp Pro Pro Phe			
530	535	540	
cct ggc gtc ttc ctg cgc ccc aag cct ctg ccc aga gta cag ctg cgg			1680
Pro Gly Val Phe Leu Arg Pro Lys Pro Leu Pro Arg Val Gln Leu Arg			
545	550	555	560

gia ccc cca cat cca cct cgg acc cag ggc tat agg acc agt ggc ccc 1728  
Val Pro Pro His Pro Pro Arg Thr Gln Gly Tyr Arg Thr Ser Gly Pro  
565 570 575

aag gtc aca gaa cta aag ccc cca gtc agg gcc cag acc agc cag gga 1776  
Lys Val Thr Glu Leu Lys Pro Pro Val Arg Ala Gln Thr Ser Gln Gly  
580 585 590

ggc cgg gag ggc cag tta cat gga cag gga ctc atg gtg ccc aca gtg 1824  
Gly Arg Glu Gly Gln Leu His Gly Gln Gly Leu Met Val Pro Thr Val  
595 600 605

gac ttg aac tcc tca gtg gaa aca cag cct gtg act tcc ttc ctg agc 1872  
Asp Leu Asn Ser Ser Val Glu Thr Gln Pro Val Thr Ser Phe Leu Ser  
610 615 620

ttg tct cag gta tcc agg cca cag ctg cca gga gag ggt gaa gaa ggg 1920  
Leu Ser Gln Val Ser Arg Pro Gln Leu Pro Gly Glu Gly Glu Glu Gly  
625 630 635 640

gag gag gat ggg gcc cca ggt gat gag gcc aca tca gaa gac agt gag 1968  
Glu Glu Asp Gly Ala Pro Gly Asp Glu Ala Thr Ser Glu Asp Ser Glu  
645 650 655

gaa gag gag gag ccg gcc gct ggg cgg ccc ctg ggt cgc tgg cgg gag 2016  
Glu Glu Glu Glu Pro Ala Ala Gly Arg Pro Leu Gly Arg Trp Arg Glu  
660 665 670

gat gcc atc aac tgg cag cgc acg ttc agc gtg ggc gcc atg gac ttc 2064  
Asp Ala Ile Asn Trp Gln Arg Thr Phe Ser Val Gly Ala Met Asp Phe  
675 680 685

gag ctc ctg cgc tct gac tgg aac gac ctg cgc tgt aac gta tcc ggg 2112  
Glu Leu Leu Arg Ser Asp Trp Asn Asp Leu Arg Cys Asn Val Ser Gly  
690 695 700

aac ctg caa ctt cct gag gcc gaa gcg gtg gat gta gtg gct cag tac 2160  
Asn Leu Gln Leu Pro Glu Ala Glu Ala Val Asp Val Val Ala Gln Tyr  
705 710 715 720

atg gag cgg cta aat gca aag cat ggc ggg cgc ttc tcg ctt cta cgc 2208  
Met Glu Arg Leu Asn Ala Lys His Gly Gly Arg Phe Ser Leu Leu Arg  
725 730 735

atc gtg aac gtg gag aag cgc cgc gac tct gca cgc ggg agc cgc ttc 2256  
Ile Val Asn Val Glu Lys Arg Arg Asp Ser Ala Arg Gly Ser Arg Phe  
740 745 750

ctc ctg gaa ctg gaa ttg caa gag cgc gga ggg agc cgc cag cgc cta 2304  
Leu Leu Glu Leu Glu Leu Gln Glu Arg Gly Gly Ser Arg Gln Arg Leu  
755 760 765

tcc gaa tac gtc ttc ctg cgg ttg ccc gga gcc cgc gtt ggg gac gaa 2352  
Ser Glu Tyr Val Phe Leu Arg Leu Pro Gly Ala Arg Val Gly Asp Glu  
770 775 780

gat gga gaa agt ccc gag ccg cct cca gcc gcc tcg atc cac cca gac 2400

56 / 75



900	905	910	
atc cac ttc cca cct aat atc ctg gac agc atc cgc aag cat tgc gtg			2784
Ile His Phe Pro Pro Asn Ile Leu Asp Ser Ile Arg Lys His Cys Val			
915	920	925	
gag ggc aag ctg gcc ttc gcc cct gtg gtc atg cgt ctg ggc tgt gga			2832
Glu Gly Lys Leu Ala Phe Ala Pro Val Val Met Arg Leu Gly Cys Gly			
930	935	940	
agc tca ccg tgg gac cca cat ggt tac tgg gaa gtg aat gga ttt ggc			2880
Ser Ser Pro Trp Asp Pro His Gly Tyr Trp Glu Val Asn Gly Phe Gly			
945	950	955	960
ctc ttt ggg atc tac aaa tca gac ttt gac aga gta gga ggc atg aac			2928
Leu Phe Gly Ile Tyr Lys Ser Asp Phe Asp Arg Val Gly Gly Met Asn			
965	970	975	
act gag gag ttc cgt gac cag tgg gga ggc gag gac tgg gaa ctt ctt			2976
Thr Glu Glu Phe Arg Asp Gln Trp Gly Gly Glu Asp Trp Glu Leu Leu			
980	985	990	
gac agg gtc ctg cag gca ggg ctg gag gtg gag agg ctt cga ctg cga			3024
Asp Arg Val Leu Gln Ala Gly Leu Glu Val Glu Arg Leu Arg Leu Arg			
995	1000	1005	
cac ttc tac cac cac tat cac tcg aag cga ggc atg tgg gcc aca cgc			3072
His Phe Tyr His His Tyr His Ser Lys Arg Gly Met Trp Ala Thr Arg			
1010	1015	1020	

agc cgc aaa ggt gcc cgc gca cag cga tcc tga

3105

Ser Arg Lys Gly Ala Arg Ala Gln Arg Ser

1025

1030

<210> 28

<211> 986

<212> PRT

<213> Mouse

<400> 28

Met Gly Ser Pro Arg Ala Ala Leu Leu Met Leu Leu Leu Arg Pro Ile

1

5

10

15

Lys Leu Leu Arg Arg Arg Phe Arg Leu Leu Leu Leu Ala Val Val

20

25

30

Ser Val Gly Leu Trp Thr Leu Tyr Leu Glu Leu Val Ala Ser Ala Gln

35

40

45

Ala Gly Gly Asn Pro Leu Asn His Arg Tyr Gly Ser Trp Arg Glu Leu

50

55

60

Ala Lys Ala Leu Ala Ser Arg Asn Ile Pro Ala Val Asp Pro Asn Leu

65

70

75

80

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85

90

95

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Pro Trp Leu Ser Glu Phe Arg Gly His Ala Asn Leu His Val Phe Glu  
115 120 125

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130 135 140

Pro Leu Tyr Pro His Ile Arg Thr Thr Leu Arg Lys Leu Ala Val Ser  
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Pro Lys Trp Thr Asn Tyr Gly Leu Arg Ile Phe Gly Tyr Leu His Pro  
165 170 175

Phe Thr Asp Gly Lys Ile Gln Phe Ala Ile Ala Ala Asp Asp Asn Ala  
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Glu Phe Trp Leu Ser Arg Asp Asp Gln Val Ser Gly Leu Gln Leu Leu  
195 200 205

Ala Ser Val Gly Lys Thr Gly Lys Glu Trp Thr Ala Pro Gly Glu Phe  
210 215 220

Gly Lys Phe Gln Ser Gln Ile Ser Lys Pro Val Ser Leu Ser Ala Ser  
225 230 235 240

Leu Arg Tyr Tyr Phe Glu Val Leu His Lys Gln Asn Asp Glu Gly Thr

245 250 255  
Asp His Val Glu Val Ala Trp Arg Arg Asn Asp Pro Gly Ala Lys Phe  
260 265 270  
Thr Ile Ile Asp Ser Pro Phe Leu Ser Leu Phe Thr Asn Glu Thr Ile  
275 280 285  
Leu Arg Met Asp Glu Val Gly His Ile Pro Gln Thr Ala Ala Ser His  
290 295 300  
Val Gly Ser Ser Asn Thr Pro Pro Arg Asp Glu Gln Pro Pro Ala Asp  
305 310 315 320  
Met Leu Arg Pro Asp Pro Arg Asp Thr Leu Phe Arg Val Pro Leu Ile  
325 330 335  
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Ser Tyr Leu Val Asp Gly Leu Pro Leu Gln Arg Tyr Gln Gly Leu Arg  
355 360 365  
Phe Val His Leu Ser Phe Val Tyr Pro Asn Asp Tyr Thr Arg Leu Ser  
370 375 380  
His Met Glu Thr His Asn Lys Cys Phe Tyr Gln Glu Ser Ala Tyr Asp  
385 390 395 400

Gln Asp Arg Ser Ser Phe Gln Glu Tyr Ile Lys Met Asp Lys Pro Glu  
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Lys His Gly Pro Glu Gln Pro Ala Gly Leu Glu Asp Gly Leu Leu Glu  
420 425 430

Glu Ser Gln Tyr Glu Asp Val Pro Glu Glu Ile Pro Thr Ser Gln Asp  
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Gln Asn Thr Gly Ile Gln Gly Arg Lys Gln Lys Thr Ile Ser Thr Pro  
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Gly Leu Gly Val Thr Asp Tyr His Leu Arg Lys Leu Leu Ala Arg Ser  
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Phe Pro Thr Arg Thr Ser Asn Ile Pro Val Gln Arg Pro Glu Lys Ser  
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515 520 525

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Lys Thr Leu Glu Gln Ser Gln Trp Leu Asn Gln Val Glu Ser Phe Ile  
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595 600 605

Glu Val Phe Glu Tyr Val Pro Met Phe Asp Pro Val Val Asn Trp Gly  
610 615 620

Gln Thr Phe Ser Ala Gln Asn Leu Asp Phe Gln Ala Leu Arg Thr Asp  
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Trp Ile Asp Leu Asn Cys Asn Thr Ser Gly Asn Leu Leu Leu Pro Glu  
645 650 655

Gln Glu Ala Leu Glu Val Thr Arg Val Phe Leu Arg Lys Leu Ser Gln  
660 665 670

Arg Thr Arg Gly Arg Tyr Gln Leu Gln Arg Ile Val Asn Val Glu Lys  
675 680 685

Arg Gln Asp Arg Leu Arg Gly Gly Arg Tyr Phe Leu Glu Leu Glu Leu  
690 695 700

Leu Asp Gly Gln Arg Leu Val Arg Leu Ser Glu Tyr Val Ser Thr Arg

705	710	715	720
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Asn Leu Gln Gly Leu Val Trp Ser Pro Arg Asn Arg His Arg His Val			
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Leu Asn Ala Gln Asp Pro Glu Pro Lys Leu Cys Trp Pro Gln Gly Phe			
	755	760	765
Ser Trp Asn His Arg Ala Val Val His Phe Ile Val Pro Val Lys Asn			
	770	775	780
Gln Ala Arg Trp Val Gln Gln Phe Ile Arg Asp Met Glu Ser Leu Ser			
785	790	795	800
Gln Val Thr Gly Asp Ala His Phe Ser Ile Ile Ile Thr Asp Tyr Ser			
	805	810	815
Ser Glu Asp Met Asp Val Glu Met Ala Leu Lys Arg Ser Arg Leu Arg			
	820	825	830
Ser Tyr Gln Tyr Leu Lys Leu Ser Gly Asn Phe Glu Arg Ser Ala Gly			
	835	840	845
Leu Gln Ala Gly Ile Asp Leu Val Lys Asp Pro His Ser Ile Ile Phe			
850	855	860	

Leu Cys Asp Leu His Ile His Phe Pro Ala Gly Ile Ile Asp Thr Ile  
865 870 875 880

Arg Lys His Cys Val Glu Gly Lys Met Ala Phe Ala Pro Met Val Met  
885 890 895

Arg Leu His Cys Gly Ala Thr Pro Gln Trp Pro Glu Gly Tyr Trp Glu  
900 905 910

Val Asn Gly Phe Gly Leu Leu Gly Ile Tyr Lys Ser Asp Leu Asp Lys  
915 920 925

Ile Gly Gly Met Asn Thr Lys Glu Phe Arg Asp Arg Trp Gly Gly Glu  
930 935 940

Asp Trp Glu Leu Leu Asp Arg Ile Leu Gln Ala Gly Leu Glu Val Glu  
945 950 955 960

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980 985

<210> 29

<211> 2961

<212> DNA

<213> Mouse



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aag ctg ctg agg agg cgc ttc cgg ctg ctg ctg ctg ctc gcc gta gta 96  
Lys Leu Leu Arg Arg Arg Phe Arg Leu Leu Leu Leu Ala Val Val  
20 25 30

tgc gtg gga ctc tgg act ctg tat ctg gag ctg gtg gcg tgc gcc cag 144  
Ser Val Gly Leu Trp Thr Leu Tyr Leu Glu Leu Val Ala Ser Ala Gln  
35 40 45

gcc gcc ggg aac ccc ctg aac cac agg tat gcc agc tgg cga gaa ctg 192  
Ala Gly Gly Asn Pro Leu Asn His Arg Tyr Gly Ser Trp Arg Glu Leu  
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gcc aag gcc cta gcc agc agg aac atc cca gcc gtt gat ccg aat ctc 240  
Ala Lys Ala Leu Ala Ser Arg Asn Ile Pro Ala Val Asp Pro Asn Leu  
65 70 75 80

caa ttc tac cgt ccc cag cgg ctg agc ctc aag gac caa gaa att gcc 288  
Gln Phe Tyr Arg Pro Gln Arg Leu Ser Leu Lys Asp Gln Glu Ile Ala  
85 90 95

cga agt agg agt agg aac agt agc tac ctg aag tgg aac aag cct gtc 336  
Arg Ser Arg Ser Arg Asn Ser Ser Tyr Leu Lys Trp Asn Lys Pro Val  
100 105 110

ccc tgg ctc tca gag ttc cgg ggc cac gcc aac cta cat gtg ttt gaa 384  
Pro Trp Leu Ser Glu Phe Arg Gly His Ala Asn Leu His Val Phe Glu  
115 120 125

gac tgg tgt ggc agc tcc atc caa cag ctg agg aac aac ctg cac ttc 432  
Asp Trp Cys Gly Ser Ser Ile Gln Gln Leu Arg Asn Asn Leu His Phe  
130 135 140

cca ctc tac ccc cac atc cgc aca act ctg agg aag ctg gct gtg tcc 480  
Pro Leu Tyr Pro His Ile Arg Thr Thr Leu Arg Lys Leu Ala Val Ser  
145 150 155 160

ccc aag tgg acc aac tat ggc ctc cgc ata ttt ggc tat ctg cac cct 528  
Pro Lys Trp Thr Asn Tyr Gly Leu Arg Ile Phe Gly Tyr Leu His Pro  
165 170 175

ttc acc gat ggg aaa atc cag ttt gcc atc gct gct gat gac aat gct 576  
Phe Thr Asp Gly Lys Ile Gln Phe Ala Ile Ala Ala Asp Asp Asn Ala  
180 185 190

gag ttc tgg ctg agt cgt gat gac cag gtc tca ggc ctt cag ctg ctg 624  
Glu Phe Trp Leu Ser Arg Asp Asp Gln Val Ser Gly Leu Gln Leu Leu  
195 200 205

gcc agc gtg ggc aag aca gga aag gaa tgg aca gcc cct gga gag ttt 672  
Ala Ser Val Gly Lys Thr Gly Lys Glu Trp Thr Ala Pro Gly Glu Phe  
210 215 220

67 / 75

Ala Lys Ser His Leu Arg His Val Leu Pro Asp Cys Pro Tyr Lys Pro  
340 345 350

agc tac ctg gtg gat gga ctc ccg cta cag cgc tac cag ggc ctc cgt 1104  
Ser Tyr Leu Val Asp Gly Leu Pro Leu Gln Arg Tyr Gln Gly Leu Arg  
355 360 365

ttt gtt cac ctg tcc ttt gtt tat ccc aat gac tat acc cgt ctg agc 1152  
Phe Val His Leu Ser Phe Val Tyr Pro Asn Asp Tyr Thr Arg Leu Ser  
370 375 380

cac atg gag acc cat aat aaa tgt ttc tac caa gaa agt gcc tat gac 1200  
His Met Glu Thr His Asn Lys Cys Phe Tyr Gln Glu Ser Ala Tyr Asp  
385 390 395 400

cag gac agg tcc agc ttc cag gaa tat atc aag atg gac aag cca gag 1248  
Gln Asp Arg Ser Ser Phe Gln Glu Tyr Ile Lys Met Asp Lys Pro Glu  
405 410 415

aag cat ggc ccg gag cag cca gca ggt ttg gag gat ggc ctt cta gaa 1296  
Lys His Gly Pro Glu Gln Pro Ala Gly Leu Glu Asp Gly Leu Leu Glu  
420 425 430

gaa tcc cag tat gaa gac gta cca gag gaa atc ccc acc tct caa gac 1344  
Glu Ser Gln Tyr Glu Asp Val Pro Glu Glu Ile Pro Thr Ser Gln Asp  
435 440 445

cag aat act ggg ata caa ggg aga aaa cag aag act att tcc acc ccg 1392  
Gln Asn Thr Gly Ile Gln Gly Arg Lys Gln Lys Thr Ile Ser Thr Pro

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ggg ctg ggt gtc act gac tac cac ctg cgg aag ctc ttg gct cgc tca			1440
Gly Leu Gly Val Thr Asp Tyr His Leu Arg Lys Leu Leu Ala Arg Ser			
465	470	475	480
cag agt ggc cct gta gcg cct ctt tcc aaa cag aac tct aca act gcc			1488
Gln Ser Gly Pro Val Ala Pro Leu Ser Lys Gln Asn Ser Thr Thr Ala			
	485	490	495
ttt cca acc agg aca agc aac atc cca gtc cag cgg cca gag aaa agc			1536
Phe Pro Thr Arg Thr Ser Asn Ile Pro Val Gln Arg Pro Glu Lys Ser			
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Asn Leu Pro Leu Ile Gln Arg Ala Arg Pro Thr Gly Asp Arg Pro Gly			
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Lys Thr Leu Glu Gln Ser Gln Trp Leu Asn Gln Val Glu Ser Phe Ile			
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Glu Val Phe Glu Tyr Val Pro Met Phe Asp Pro Val Val Asn Trp Gly	
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625 630 635 640	
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Gln Glu Ala Leu Glu Val Thr Arg Val Phe Leu Arg Lys Leu Ser Gln	
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Leu Asp Gly Gln Arg Leu Val Arg Leu Ser Glu Tyr Val Ser Thr Arg	
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ggc tgg cgg gga ggt gac cac cca ggc agg gag gac aca gaa gct cgg	2208
Gly Trp Arg Gly Gly Asp His Pro Gly Arg Glu Asp Thr Glu Ala Arg	
725	735
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Asn Leu Gln Gly Leu Val Trp Ser Pro Arg Asn Arg His Arg His Val	
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Leu Asn Ala Gln Asp Pro Glu Pro Lys Leu Cys Trp Pro Gln Gly Phe	
755	765
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Ser Trp Asn His Arg Ala Val Val His Phe Ile Val Pro Val Lys Asn	
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Gln Ala Arg Trp Val Gln Gln Phe Ile Arg Asp Met Glu Ser Leu Ser	
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820	825	830	
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835	840	845	
ctg cag gct ggc ata gac ctg gtg aag gat cca cac agc atc atc ttc Leu Gln Ala Gly Ile Asp Leu Val Lys Asp Pro His Ser Ile Ile Phe			2592
850	855	860	
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865	870	875	880
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885	890	895	
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915	920	925	



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Ile Gly Gly Met Asn Thr Lys Glu Phe Arg Asp Arg Trp Gly Gly Glu

930

935

940

gac tgg gag ctg ctg gac agg att ctc caa gca ggc ctg gaa gtg gag 2880  
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945

950

955

960

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965

970

975

atg tgg aac cgt cgc caa atg aag atg ccg tga 2961  
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980

985

&lt;210&gt; 30

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: FLAG peptide

&lt;400&gt; 30

Asp Tyr Lys Asp Asp Asp Asp Lys

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<210> 31

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer used in PCR for amplifying  
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<212> DNA

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<213> Artificial Sequence

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<210> 34

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<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide primer used in PCR for amplifying  
mNGalNAc-T1 cDNA

<400> 34

ggaattctca cggcattctc atttggcga 29

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/10309

## A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl<sup>7</sup> C12N 15/54, 9/10, 5/10, C12Q 1/68, C07K 16/40, G01N 33/53, 33/566, 33/574  
//C12P 21/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl<sup>7</sup> C12N 15/00-15/90, 9/00-9/99, 5/10, C12Q 1/68, C07K 16/40Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
Japanese Utility Model Gazette 1926-1996, Japanese Publication of Unexamined Utility Model Applications 1971-2001, Japanese Registered Utility Model Gazette 1994-2001, Japanese Gazette Containing the Utility Model 1996-2001

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS/WPI (DIALOG), MEDLINE (STN), JSTPlus (JOIS)  
PIR/SwissProt/GeneSeq, DDBJ/EMBL/GenBank/GeneSeq

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 01/53524 A2 (THE NOTTINGHAM TRENT UNIVERSITY) 2001.07.26 & EP 1250457 A2	15 1-14, 16-19
A	WO 01/90369 A1 (SHANGHAI BIOWINDOW GENE DEVELOPMENT INC.) 2001.11.29 & CN 1323895 A	1-19
A	JP 2001-165933 A (TAIHO PHARM.CO., LTD.) 2001.06.22 (NO FAMILY)	1-19



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;"

document member of the same patent family

Date of the actual completion of the international search

12.11.03

Date of mailing of the international search report

25.11.03

Name and mailing address of the ISA/JP

Japan Patent Office

3-4-3, Kasumigasaka, Chiyoda-ku, Tokyo 100-8915, Japan

Authorized officer

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Telephone No. +81-3-3581-1101 Ext. 3488



4N

2937

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NEELEMAN A.P.et al. alpha-Lactalbumin affects the acceptor specificity of Lymnaea stagnalis albumen gland UDP-GalNAc:GlcNAcbeta-R beta1-4-N-acetylgalactosaminyltransferase: Synthesis of GalNAcbeta1-4Glc. Proc.Natl.Acad.Sci.USA 1996, Vol.93, p.10111-10116	1-19
A	GOTOH M.et al. Enzymatic Synthesis of Chondroitin with a Novel Chondroitin Sulfate N-Acetylgalactosaminyltransferase That Transfers N-Acetylgalactosamine to Glucuronic Acid in Initiation and Elongation of Chondroitin Sulfate Synthesis. J.Biol.Chem. Oct.11,2002 (Epub Aug.5,2002), Vol.277, No.41, p.38189-38196	1-19
A	KAWAR Z.S.et al. Molecular Cloning and Enzymatic Characterization of a UDP-GalNAc:GlcNAcbeta-R beta-1,4-N-Acetylgalactosaminyltransferase from Caenorhabditis elegans. J.Biol.Chem. Sept.20,2002 (Epub Jul.11,2002), Vol.277, No.38, p.34924-34932	1-19

# INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/JP03/10309**

## Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 20-23  
because they relate to subject matter not required to be searched by this Authority, namely:  
The subject matter of claim 20-23 relates to [diagnostic methods practiced on the human body], which does not require an intentional search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and [Rule 39.1(iv)].
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The present application is including two inventions which are the isolated proteins called NGalNac-T1 and NGalNac-T2, and the feature common to them is an isolated protein having the activity of transferring N-acetylgalactosamine to N-acetylglucosamine via a beta 1-4 linkage. However, the search has revealed that this protein is not novel since it is electronically disclosed by KAWAR Z.S. et al. (in J. Biol. Chem. Jul. 11, 2002, Vol. 277, No. 38, p. 34924-34932). Since there exists no other common feature which can be considered as a special technical feature within the meaning of PCT Rule 13.2, second sentence, no technical relationship within the meaning of PCT Rule 13 between the different inventions can be seen.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
claim 1-19 related to NGalNac-T1

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☒

No protest accompanied the payment of additional search fees.